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Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.01/A1

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH R01 MH093595 03

NIH R21 OD016562 01

HHMI Investigator

Title: Developmental origin and dynamics of Hoxb8 microglia

Authors: *D. VAN DEREN, JR, B. XU, M. ECONOMO, M. WACHOWIAK, P. TVRDIK, G. SPANGRUDE, M. CAPECCHI;
Univ. of Utah, Salt Lake City, UT

Abstract: Microglia are the innate immune cells that constantly survey the environment of the central nervous system (CNS). Recently, it was demonstrated that microglia originate from yolk sac-derived macrophages during primitive hematopoiesis and subsequently infiltrate the embryonic brain (Ginhoux et al. 2010). However, it is unclear whether this group accounts for the total adult microglia population following development. We have addressed this question using a Hoxb8-IRES-Cre reporter mouse line because the Hoxb8 lineage includes a subset of microglia that only appear postnatally in the brain parenchyma (Chen et al. 2010). Using the Hoxb8-IRES-Cre mouse line to report cell lineage, we show that a substantial population (~30%) of postnatal cortical microglia arises independently of the yolk sac, during definitive hematopoiesis. We performed FACS analysis of the yolk sac and anterior and posterior poles of the embryos harvested from Hoxb8-IRES-Cre+/-; Rosa26-tdTomato (Ai14)+/-; Cx3cr1-GFP+/- triple transgenic mice at embryonic (E) stages E8.5 - E10.5. Our data show that less than 10% of CD45+ cells in the yolk sac during the critical stage of primitive macrophage formation (E8.5) are positive for the Hoxb8 cell lineage (Hoxb8+). From our analysis, it also appears that Hoxb8+ cells arise earlier than Cx3cr1+ cells, and are of distinct origins initially. The CD45+ Hoxb8+ cells are mostly c-kit positive at this stage, indicating that this population represents hematopoietic progenitor cells. During definitive hematopoiesis, the percentage of Hoxb8+ cells rises continuously and accounts for 100% of the peripheral blood and bone marrow cells, and about 40-50% of cortical microglia in the adult brain parenchyma. We have established that the bulk of infiltration of Hoxb8+ microglia in the brain occurs during the first postnatal week. Using live 2-photon microscopy, we delineated their transition from the meningeal space to the

neonatal cortex. While both Hoxb8+ and Hoxb8- microglia appear to have similar motility and speed of their processes, their response to neonatal brain injury is more exaggerated in the Hoxb8+ population. This study provides evidence for a separate class of microglia, Hoxb8+ microglia, which derives from definitive, not primitive, hematopoiesis and infiltrates the brain postnatally.

Disclosures: D. Van Deren: None. **B. Xu:** None. **M. Economo:** None. **M. Wachowiak:** None. **P. Tvrdik:** None. **G. Spangrude:** None. **M. Capecchi:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.02/A2

Topic: A.02. Neurogenesis and Gliogenesis

Support: MH093595

Title: Hoxb8-positive microglia have distinct developmental and physiological properties

Authors: *S. DE[1], N. NAGARAJAN[1], B. XU[1,3], E. PEDEN[1], P. TVRDIK[1,2], M. CAPECCHI[1,2,3];
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Abstract: *Hoxb8* mutant mice exhibit an excessive grooming behavior which is manifested by localized hair removal and self-inflicted skin lesions, a behavior similar to the OCD-spectrum disease trichotillomania (Chen et al., 2010; Greer and Capecchi, 2002). Pathological grooming, which depends on specific brain circuits, can be restored in *Hoxb8* deficient mice to a normal grooming pattern following wild type bone marrow transplantations, suggesting hematopoietic origin of this behavior. The predominant cells in the brain derived both from the *Hoxb8* lineage are certain microglia, designated as the "*Hoxb8*$^{+}$ microglia". We are interested in elucidating the specific roles of *Hoxb8* microglia in neural circuits. For unequivocal cell identification, we used *Hoxb8*-IRES-Cre$^{+/-}$; *Rosa26*-tdTomato (Ai14) $^{+/-}$; *Cx3cr1*-GFP$^{+/-}$ triple transgenic mouse model, in which all microglia are labeled with GFP, and the *Hoxb8*$^{+}$ microglia are also labeled with tdTomato. We show that the *Hoxb8* microglia populate the brain parenchyma after birth with a peak density around postnatal day 8 (P8). As determined by EdU incorporation, proliferative rates in both *Hoxb8*$^{-}$ and *Hoxb8*$^{+}$ populations are relatively high during the first postnatal week, and similar in both subtypes. This indicates that the increase in the relative *Hoxb8*$^{+}$ population density is due to increased cortical infiltration rather than selective proliferative advantage.

Interestingly, *Hoxb8*$^{+}$ display several morphological differences compared to *Hoxb8*$^{-}$ microglia at several developmental stages, such as greater overall length of the projections and branching density on individual projections. When challenged in an injury model (induced by facial nerve axotomy), the non-*Hoxb8* microglia show higher degree of activation compared to the *Hoxb8* cells. Our data suggests that *Hoxb8*$^{+}$ microglia have unique functions, which cannot be compensated by the non-*Hoxb8* microglia. Elucidating the differences between the *Hoxb8* and non-*Hoxb8* population will help to identify specific roles that contribute to the mutant behavior.

Disclosures: S. De: None. **N. Nagarajan:** None. **B. Xu:** None. **E. Peden:** None. **P. Tvrdik:** None. **M. Capecchi:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.03/A3

Topic: A.02. Neurogenesis and Gliogenesis

Support: DFG SFB/TR3

DFG STE 552/3

EU FP7-202167 NeuroGLIA

EU ESF EuroEPINOMICS

Title: New function of synapses: Synaptic input as a directional cue for migrating interneuron precursors

Authors: *A. K. WEFERS[1,2], C. HABERLANDT[2], J. J. L. VAN DER WANT[3], C. STEINHÄUSER[2], K. SCHILLING[1], R. JABS[2];
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Abstract: During CNS development, interneuron precursors have to migrate extensively before they can integrate in their specific microcircuits. The mechanisms that assure interneuron dispersal and interneuron/projection neuron matching during histogenesis remain largely elusive. We combined time-lapse video-microscopy and electrophysiological analysis of the nascent cerebellum to address this issue. We found that cerebellar interneuronal precursors regularly show spontaneous postsynaptic currents, indicative of synaptic innervation, well before settling

in the molecular layer. Ablation of synaptic communication by blocking vesicular release in acute slices of developing cerebella slows migration and impedes the directionality of interneuronal precursors in transit. These results establish an unprecedented early synaptic innervation of migrating neuronal precursors and demonstrate a role for synapses in the regulation of migration and pathfinding.

Disclosures: A.K. Wefers: None. **C. Haberlandt:** None. **J.J.L. van der Want:** None. **C. Steinhäuser:** None. **K. Schilling:** None. **R. Jabs:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.04/A4

Topic: A.02. Neurogenesis and Gliogenesis

Support: ANR-2010-JCJC-1404-01

Appel a Projets du Conseil Scientifique de l'Université Lille 2

Title: Origin and migration of GnRH neurons in early human embryonic development

Authors: *F. CASONI[1], F. LUZZATI[2], F. COLLIER[3], V. PREVOT[4], P. GIACOBINI[4]; [1]Inserm, Jean-Pierre Aubert Res. Center, U837, Lille, France; [2]Neurosci. Inst. Cavalieri Ottolenghi, Torino, Italy; [3]Gynaecology Service, Ctr. Hospitalier of Lille, Hosp. Jeanne de Flandre, Lille, France; [4]INSERM, Jean-Pierre Aubert Res. Center, U837, Lille, France

Abstract: Gonadotropin-Releasing Hormone (GnRH) neurons are the central regulators of sexual development and reproduction in all vertebrates. During embryonic development, GnRH neurons differentiate in and migrate from the vomeronasal organ (VNO) to the ventral forebrain where they will later become integral members of the hypothalamic-pituitary-gonadal axis. Although different studies have shown that GnRH mature similarly in different vertebrates, the embryonic development of GnRH cells in human embryonic forebrain has not been fully characterized. Here we have examined the initial stages of GnRH cell differentiation, the chemical makeup of the migratory route and the intracerebral distribution of GnRH neurons in the embryonic human telencephalon during the first trimester of gestation. For the first time, we described subpopulations of neurons and ensheathing cells emerging from the olfactory placode by 5 weeks and half of gestation, which precede the emergence of the GnRH neurons and olfactory axons. Using immunohistochemistry and 3D-reconstruction analysis, we provide evidence that the development of the human GnRH system begins earlier than previously

thought and we characterize a new intracerebral migratory pathway of GnRH neurons never described in other species. Finally, we performed a quantitative analysis of GnRH cell distribution throughout the entire migratory route and provide evidence that early human foetuses contain a complement of GnRH cells much greater than previously thought.

Disclosures: F. Casoni: None. **F. Luzzati:** None. **F. Collier:** None. **V. Prevot:** None. **P. Giacobini:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.05/A5

Topic: A.02. Neurogenesis and Gliogenesis

Title: Negr1 is required for transition of migrating pyramidal neurons from layer V to layer II/III of the mouse cerebral cortex

Authors: *J. SZCZURKOWSKA[1], F. PISCHEDDA[2], M. SCHÄFER[3], G. PICCOLI*[2], L. CANCEDDA*[1];
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Abstract: The mammalian cerebral cortex is a remarkably complex structure with unique laminar organization. The establishment of a functional neural circuitry in the cerebral cortex requires this lamination, which is achieved through directional migration of neurons during perinatal development. Indeed, newborn pyramidal neurons migrate along radial glia fibers, in an inside-out manner to create the six-layered structure of the neocortex. Improper neural migration can lead to cell death and/or ectopic positioning with functional consequences on proper wiring in the neuronal network. Common knowledge indicates cell adhesion molecules (CAMs) as essential for proper neural migration.
Neuronal growth regulator 1 (Negr1) protein is a member of IgLON family belonging to immunoglobulin superfamily of CAMs. Negr1 is highly expressed in the cerebral cortex, hippocampus and cerebellum, and its expression increases gradually during postnatal development. Interestingly, NEGR1 gene mutations have been recently associated to autism, a neurodevelopmental disorder characterized by defective neuronal circuit formation and sensory abnormalities. So far, few in vitro studies have suggested a role for Negr1 in neurite outgrowth, but nothing is known about Negr1 function in in vivo neurodevelopment. To address this issue,

we took advantage of the in utero electroporation technique to acutely alter the expression levels of Negr1 in the developing cerebral cortex in vivo. We downregulated Negr1 expression by RNA interference (siRNA) in late-born pyramidal neurons, and found that Negr1 siRNA caused defects in radial migration of these neurons to the superficial layers of the neocortex. In particular, we found ectopic positioning of a number of Negr1 siRNA-expressing neurons. Interestingly, these ectopic neurons were located almost exclusively at the border between layer 5 and layer 4, and this effect was restricted only to the somatosensory cortex.
Taken together, these data suggest that Negr1 is necessary for proper neuronal migration of pyramidal neurons in the somatosensory cortex, indicating a possible role for this complex in neurodevelopmental disorders such as autism.
*: equal contribution

Disclosures: J. Szczurkowska: None. **F. Pischedda:** None. **M. Schäfer:** None. **G. Piccoli*:** None. **L. Cancedda*:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.06/A6

Topic: A.02. Neurogenesis and Gliogenesis

Support: Stowers Institute

NSF Grant IOS-0719251

Title: Dorsal migration and formation of the secondary, permanent chain of sympathetic ganglia as revealed by confocal time-lapse analysis in chick

Authors: *J. C. KASEMEIER[1], F. LEFCORT[3], P. M. KULESA[2];
[1]Kulesa Lab., [2]Stowers Inst., Kansas City, MO; [3]Cell Biol. and Neurosci., Montana State Univ., Bozeman, MT

Abstract: The sympathetic nervous system (SNS) plays a vital role in maintaining homeostasis. In conjunction with the parasympathetic nervous system, all involuntary, autonomic functions of the vertebrate organism are regulated, including breathing, blood flow and heart rate. All sympathetic neurons differentiate from the trunk neural crest, a pluripotent, heterogeneous cell population that gives rise to the vast majority of the peripheral nervous system, including dorsal root ganglia, sympathetic and parasympathetic ganglia and enteric nervous system. After arriving at the dorsal aorta, neural crest cells respond to cues secreted by the dorsal aorta, primarily BMP-

4, that induce their differentiation to sympathetic neurons. Strikingly, primary sympathetic ganglia (SG) form as discrete transient structures in a chain along the vertebrate axis. Within hours of their formation, sympathetic precursors migrate dorsally towards the ventral surface of the DRG, and form the permanent, secondary chain of SG. The cellular and molecular mechanisms that mediate the dorsal migration and formation of the secondary SG are largely unknown, most likely due to the ventral location deep within the embryo. Using transverse slice culture and confocal time-lapse microscopy, we detail the events involved in secondary SG formation. Interestingly, the primary SG move as a cohesive cluster during their dorsal migration, and use extensive cell-cell contacts among SG cells before re-condensing into ganglia. Filopodial extensions from migrating sympathetic neurons span over 100um in length and contact the forming spinal nerve. Tissue ablation of the spinal nerve results in disrupted dorsal migration. Repositioning of the spinal nerve leads to filopodial extensions from SG cells entering known inhibitory regions to interact with the newly positioned spinal nerve. These data indicate cell-cell and cell-environmental interactions are important for proper SG positioning and development.

Disclosures: J.C. Kasemeier: None. **F. Lefcort:** None. **P.M. Kulesa:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.07/A7

Topic: A.02. Neurogenesis and Gliogenesis

Support: Swedish Research Council

Tobias Stiftelsen

Knut och Alice Wallenbergs Stiftelse

the ERC

Title: Dynamics of oligodendrocyte turnover and myelination in humans

Authors: *M. YEUNG[1], M. SALEHPOUR[2], S. ZDUNEK[1], S. BERNARD[3], O. BERGMANN[1], K. ALKASS[4], G. POSSNERT[2], H. DRUID[4], L. BRUNDIN[5], J. FRISÉN[1];
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Abstract: Oligodendrocytes are the myelinating cells of the CNS and are essential for proper brain function as they produce the myelin sheath, which is critical for fast axonal conduction and survival of the axons. Myelination has been observed to be modulated by experience and believed to contribute to neural plasticity. Although lost myelin can be regenerated, this remyelination process is often incomplete, resulting in neurological impairment. Studies in experimental animals have shown a continuous generation of oligodendrocytes in the adult brain. However, to what extent this event occurs in the adult human brain remains elusive as methods employed in experimental animals, such as paradigms with labeled nucleotide analogs are not possible to apply in humans.
We have assessed the dynamics of oligodendrocyte generation and myelination in the corpus callosum of the human brain. By measuring the integration of 14C, derived from nuclear bomb testing during the Cold War, in genomic DNA, we found that there is addition, and a small exchange, of oligodendrocytes until myelination is completed in early adulthood. The prospect of ongoing oligodendrogenesis is of particular interest as this is the main cell population affected in multiple sclerosis. Consequently, understanding the occurrence of generation of oligodendrocytes in the physiological and pathological brain may further our knowledge regarding tissue homeostasis and disease pathologies. Furthermore, the occurrence of such event may be a potential target for establishing new therapeutic intervention aiming at affecting this process in brain pathologies.

Disclosures: M. Yeung: None. **M. Salehpour:** None. **S. Zdunek:** None. **S. Bernard:** None. **O. Bergmann:** None. **K. Alkass:** None. **G. Possnert:** None. **H. Druid:** None. **L. Brundin:** None. **J. Frisén:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.08/A8

Topic: A.02. Neurogenesis and Gliogenesis

Support: NS46616 (JAG)

Title: Mitochondrial manipulations interfere with interneuron migration

Authors: *E. G. LIN-HENDEL[1,4,2], G. CHO[6], M. J. MCMANUS[5], D. C. WALLACE[5], S. A. ANDERSON[3,4], J. A. GOLDEN[6];

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Abstract: Patients with mitochondriopathies can present with epilepsy and autism: both disorders in which cortical interneuron dysfunction is implicated. The early onset of autistic or epileptic features in children with mitochondrial disease along with the association of these features with interneuron dysfunction suggest mitochondria may play essential roles in interneuron development. Tangential migration of interneurons from the basal forebrain into the overlying cerebral cortex is likely to be an energetically demanding process, however little is known regarding a possible role for mitochondria in neuronal migration. We hypothesized that mitochondrial function is crucial to interneuron migration, and that this function influences tangential migration to a greater extent that the radial glial guided migration of cortical projection neurons. To test this hypothesis, we used a combination of explant, and organotypic slice culture in the murine developing nervous system to investigate function of mitochondria in normal interneuron migration.
Immunohistochemistry and live imaging data suggest that mitochondria cluster in specific subcellular regions of migrating interneurons. We find this localization is dynamic depending on the cell's migrational morphology. Pharmacologic and genetic targeting of Adenosine nucleotide transporter 1 and 2 (Ant1, Ant2) in migrating interneurons result in abnormal trailing process behavior, shifts in centrosome position, and reduced migration, indicating a role for mitochondria in rear retraction and polarity, and that mitochondrial function is essential for interneuron migration. Expression of a dominant negative form of Miro1, a molecular motor adaptor allowing for mitochondrial movement in migrating interneurons results in reduced migration rates, but cause increased direction changes, reducing their ability to reach the cortex. These data suggest that interneuron migration is sensitive to perturbations in mitochondrial metabolism, and provide a potential pathogenesis for the neurocognitive phenotypes found in various mitochondrial disorders.

Disclosures: E.G. Lin-Hendel: None. **G. Cho:** None. **M.J. McManus:** None. **D.C. Wallace:** None. **S.A. Anderson:** None. **J.A. Golden:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.09/A9

Topic: A.02. Neurogenesis and Gliogenesis

Support: CREST

KAKENHI 25123722

Title: Excitatory cortical neurons are classified into two distinct groups according to their initial axonal direction

Authors: *Y. HATANAKA[1,2], K. YAMAUCHI[3], T. NAMIKAWA[4], Y. KAWAGUCHI[1,2];
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Abstract: Excitatory cortical neurons are output neurons whose axons project subcortically or intracortically (i.e. ipsilateral cortico-cortical and callosal projection), although there are some exceptions such as certain local-circuit neurons. Basically, subcortially projecting neurons are located in deep layers, intracortically projecting neurons are located in upper layers and to some extent in deep layers, and local-circuit neurons are located between these two layers. Recently, we have shown that embryonic day (E) 12.5 labeled ventricular zone (VZ) cell-progeny, which give rise to deep layer neurons, initiate directed axon outgrowth in the intermediate zone (IZ) before radial migration into the cortical plate (CP) (Hatanaka and Yamauchi, Cereb Cortex 23,105, 2013). This observation prompted us to examine whether all excitatory cortical neurons initiate directed axon outgrowth in the IZ, and if so, how initial axonal extension is associated with the mature projection pattern. To address this question, we labeled mouse VZ cells in the lateral cortex at several developmental stages (from E11.5 to E15.5) by *in utero* electroporation, and examined axonal formation by their progeny, as well as marker expression and the layer distribution of labeled cells at later stages. We found that all labeled CP cells and many IZ cells exhibited long tangential processes in the IZ. Notably, these processes only extended either laterally or medially, and their direction was age-dependent: the vast majority of the processes derived from earliest emigrants from E12.5 or earlier VZ progeny extended laterally, while those from E13.5 or later extended medially toward the corpus callosum. Marker expression of the labeled cells was consistent with the idea that the former is a subcortical projection population and the latter a callosal projection population. At postnatal 3 weeks, E12.5 labeled cells were distributed mainly in deep layers, while E13.5 or later labeled cells were distributed in upper layers including layer IV and to some extent in deep layers. These results indicate that excitatory cortical neurons share a common trait of directional axon outgrowth in the IZ, and that they can be classified into two distinct groups according to their axonal direction; a subcortical (lateral) projection group, which develops axons earlier, and a callosal (medial) projection group, including intracortical projection neurons in deep layers, local circuit neurons in layer IV, and neurons in upper layers. Although some of them may prune their initial projections during

maturation, it seems that the formation of callosal projections is the default mode of the latter group.

Disclosures: Y. Hatanaka: None. **Y. Kawaguchi:** None. **K. Yamauchi:** None. **T. Namikawa:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.10/A10

Topic: A.02. Neurogenesis and Gliogenesis

Title: Sp9 is required for the normal migration of cortical interneurons

Authors: *Q. ZHANG, Y. ZHANG, Z. LIU, J. LI, Z. YANG;
Inst. of Brain Sci. ,Fudan Univ., Shanghai, China

Abstract: The neocortex contains two main neuron populations, the excitatory glutamatergic projection (pyramidal) neurons and the GABAergic inhibitory interneurons, which constitute about 20% of all neocortical neurons. Neocortical projection neurons are born during embryogenesis in the ventricular zone of the dorsal pallium (neocortex), whereas neocortical interneurons are derived from the subpallium (including the MGE and LGE/CGE) and reach the neocortex by tangential migration. Dlx1/2 homeobox genes are essential for the tangential migration of subpallial-derived GABAergic interneurons to neocortex. However, it remains largely unknown the mechanisms underlying this process. Here, we show that Sp9, a zinc finger gene, is expressed in most (perhaps all) postmitotic GABAergic neurons of the MGE and LGE/CGE. Sp9 is down-regulated when interneurons migrate into the neocortex, therefore mature interneurons in the neocortex do not express Sp9. Interneurons and medium spiny neurons in the neonatal striatum also express Sp9; however, Sp9 is not expressed in the adult striatum. We also show that Sp9 is required for the normal tangential migration of neocortical GABAergic interneurons.

Disclosures: Q. Zhang: None. **Y. Zhang:** None. **Z. Liu:** None. **J. Li:** None. **Z. Yang:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.11/A11

Topic: A.02. Neurogenesis and Gliogenesis

Support: CONACyT Fellowship number 244955

CINVESTAV

Title: Cytoskeleton arrangement, associated proteins and their relationship with the migration/invasion pattern of culture pituitary adenoma cells

Authors: *D. AVILA[1], M. MENDOZA[1], A. ORTIZ-PLATA[2], E. AGUIRRE-BENÍTEZ[3], M. GONZÁLEZ DEL PLIEGO[3];
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Abstract: Introduction
Pituitary adenomas are generally considered benign, slow-growing and rarely metastasize, but some cells may migrate infiltrating adjacent tissues. Cell migration requires coordinated communication between the actin cytoskeleton, intermediate filaments and tubulin cytoskeleton.
Objective
Characterize the arrangement of the cytoskeleton, adhesion proteins and the relationship of the cytoskeleton arrangement with the migration patterns that the pituitary adenoma cells could adopt.
Methods
Ten macroadenomas were studied, seven invasive and 3 non-invasive. The tumor tissues were donated by the National Institute of Neurology and Neurosurgery, in Mexico City. After surgical removal, a cell suspension was obtained using an enzymatic-mechanical method and the suspended cells were cultured in a three-dimensional system of alginate gel pearls.. The cells were liberated from the alginate gel pearls and were cultured on cover glasses for further immunocytochemical analysis.
Results
We found that cells macroadenomas take two main arrangements of the cytoskeleton: discontinuous cortical rings and a fine mesh, and in uncured filaments in membrane protrusions with small stress fibers, arrangement compatible with the amoeboid-like migration pattern. They arrange their microtubules as a diffuse network, suggesting a deficiency in their secretory activity and in migration directionality. With regard to the intermediate filaments, all cells expressed vimentin and only a few expressed GFAP; both intermediate filaments typically are not expressed in normal secretory cells. These findings suggest that cells acquire a higher elastic

capacity. Interestingly, all the different tumor cells showed poor substrate adhesion. All macroadenomas cells expressed the N-cadherin in cell-cell contact sites and wide-spread in the cytoplasm.
Conclusions
Taken together, these results show that macroadenomas cells organize their cytoskeleton in two ways, as leukocytes, and as mesenchymal cells with low substrate adhesion, besides their invasive capacity. In conclusion invasive-silent-macroadenoma cells in culture adopt a rounded shape with low substrate adhesion and showed tubulin cytoskeleton disorganization suggesting an amoeboid-like migration pattern.

Disclosures: D. Avila: None. **M. Mendoza:** None. **A. Ortiz-plata:** None. **E. Aguirre-Benítez:** None. **M. González del Pliego:** None.

Poster

509. **Cell Migration: Cellular Dynamics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 509.12/A12

Topic: A.02. Neurogenesis and Gliogenesis

Support: Ministry of Education, Science, Technology, Sports and Culture of Japan

Title: Biochemical and morphological characterization of A2BP1 in the neuronal tissue

Authors: *K.-I. NAGATA, N. HAMADA, H. ITO, I. IWAMOTO, M. MIZUNO, R. MORISHITA, Y. INAGUMA, H. TABATA;
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Abstract: Background: Ataxin-2-binding protein 1 (A2BP1), which is also termed FOX1 or RBFOX1, is considered to regulate alternative splicing of important neuronal transcripts and has been implicated in neurological/developmental disorders such as autism and mental retardation. In this study, we prepared a specific antibody against A2BP1, anti-A2BP1, and carried out biochemical and morphological analyses of A2BP1 in rat and mouse neuronal tissues.
Results: We prepared a rabbit polyclonal antibody, anti-A2BP1, against full length of mouse A2BP1-A016 isoform, and affinity-purified it. Specificity of the antibody was confirmed with COS7 cell lysates expressing Myc-A2BP1. Anti-A2BP1 recognized both A016 and A030 isoforms in western blotting and detected endogenous A2BP1 in rodent brain tissues during developmental stages. Biochemical fractionation clarified that A2BP1 with ~60 kDa and ~50 kDa were relatively enriched in the synaptosomal and postsynaptic density fractions, respectively. In immunohistochemical analyses, A2BP1 was detected mainly in the nucleus and

weakly in the cell body and dendrites of excitatory neurons in rodent cerebral cortices. In contrast, A2BP1 was not observed in mitotically active progenitor cells in the developing cortices. We then performed immunofluorescence analyses using primary cultured rat hippocampal neurons. In immature 3div neurons, A2BP1 was evenly expressed in the soma, axons and dendrites. Then, as differentiation advances, A2BP1 distribution gradually changed and it came to be enriched in the nucleus. Interestingly, A2BP1 was also observed adjacent to a presynaptic protein, synaptophysin, and a postsynaptic protein, PSD-95, in 20 div neurons. Conclusion: In this study, we carried out biochemical and morphological analyses of A2BP1 by the use of a homemade antibody. The results obtained suggest the role of A2BP1 in the nucleus and near excitatory synapses of differentiated neurons.

Disclosures: K. Nagata: None. **N. Hamada:** None. **H. Ito:** None. **I. Iwamoto:** None. **M. Mizuno:** None. **R. Morishita:** None. **Y. Inaguma:** None. **H. Tabata:** None.**Poster**

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.01/A13

Topic: A.02. Neurogenesis and Gliogenesis

Support: Chatholic University D3.2 Funds

Title: Modulation of mouse hippocampal neurogenesis by cyclic nucleotide-gated channels

Authors: M. V. PODDA[1], R. PIACENTINI[1], S. A. BARBATI[1], A. MASTRODONATO[1], D. PUZZO[2], *M. D'ASCENZO[1], L. LEONE[1], C. GRASSI[1];
[1]Inst. of Human Physiol., Catholic Univ., Rome, Italy; [2]Dept. of Bio-Medical Sci., Univ. of Catania, Catania, Italy

Abstract: Throughout life adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that play critical roles in learning and memory. There is great interest in identifying physiological and exogenous stimuli able to stimulate this endogenous process because of their potential use for treatment of neurodegenerative diseases entailing cognitive impairment. Aim of the present study was to ascertain the possible role of cyclic nucleotide-gated (CNG) channels in hippocampal neurogenesis.These voltage-independent channels activated by cyclic nucleotides, first described in retinal and olfactory receptors, have been receiving increasing attention for their involvement in several brain functions. We found that the rod-type, CNGA1, and olfactory-type, CNGA2, subunits are expressed in hippocampal neural stem cells (NSCs) in culture as well as in the hippocampal neurogenic niche of adult mice. Pharmacological blockade of CNG channels did not affect NSC proliferation but reduced their differentiation toward the neuronal

phenotype. Addition of 8-Br-cGMP (1 mM) to the culture medium enhanced neuronal differentiation of NSCs and this effect was prevented by CNG channel blockade. In addition, patch-clamp recordings from neuron-like differentiating NSCs revealed cGMP-activated currents that are reminiscent of those flowing through CNG channels. Taken together these data provide novel insights into the role of CNG channels in promoting hippocampal neurogenesis, which may prove to be relevant for stem cell-based treatment of cognitive impairment and brain damage.

Disclosures: M.V. Podda: None. **R. Piacentini:** None. **S.A. Barbati:** None. **A. Mastrodonato:** None. **D. Puzzo:** None. **M. D'Ascenzo:** None. **L. Leone:** None. **C. Grassi:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.02/A14

Topic: A.02. Neurogenesis and Gliogenesis

Support: Deutsche Forschungsgemeinschaft

BMBF

Bonfor

Title: Role of γRIMs in the regulation of neuronal arborization

Authors: *K. MICHEL[1], A.-M. OPRISOREANU[1], E. ALVAREZ-BARON[1], C. HOOGENRAAD[2], S. FRANKEN[3], A. BECKER[1], S. SCHOCH[1];
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Abstract: The electrical properties of neuronal cells are shaped by their dendritic morphology and the composition and density of their synapses. Therefore, the mechanisms underlying dendritic morphogenesis have been extensively studied and have implicated several synaptic proteins to play a role in the regulation of neuronal branching. By analyzing primary rat neurons with reduced expression of RIM3γ and RIM4γ we recently identified the two short members of the RIM protein family as key regulators of neuronal arborization (Alvarez-Baron, Michel et al., J. Neurosci., 2013). The large multidomain RIMs, RIM1 and RIM2, are centrally involved in the organization of the presynaptic active zone. As a first step into gaining further insight into the γRIM-dependent processes mediating neurite outgrowth we performed a tandem-

affinity/Massspectrometry screen to identify γRIMs-specific interacting proteins. In this screen we found a cluster of proteins involved in the regulation of cytoskeleton and transport processes during neuronal development. For RIM3γ in particular, we uncovered proteins acting as Ca^{2+} sensors and nuclear transport receptors, suggesting an isoform specific function apart from neuronal outgrowth. The results of this study suggest a role for γRIMs in the transport processes delivering membrane and proteins to site of neuronal outgrowth.

Disclosures: K. Michel: None. **A. Oprisoreanu:** None. **E. Alvarez-Baron:** None. **C. Hoogenraad:** None. **S. Franken:** None. **A. Becker:** None. **S. Schoch:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.03/A15

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant RO1NS039007

NIH Grant RO1MH071679

Title: Prox1 regulates the migration and maturation of caudal ganglionic eminence-derived cortical interneurons

Authors: *G. MIYOSHI[1], A. YOUNG[1], T. KARAYANNIS[1], M. MCKENZIE CHANG[1], T. PETROS[1], A. LAVADO[2], T. IWANO[3], H. TANIGUCHI[4], M. NAKAJIMA[5], J. Z. HUANG[4], N. HEINTZ[5], F. MATSUZAKI[3], G. OLIVER[2], R. MACHOLD[1], G. FISHELL[1];
[1]New York Univ. SoM, NEW YORK, NY; [2]St. Jude Children's Res. Hosp., Memphis, TN; [3]RIKEN Ctr. for Developmental Biol., Kobe, Japan; [4]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [5]The Rockefeller Univ., New York, NY

Abstract: The vast majority of GABAergic neocortical interneurons arise from the medial (~70%) and caudal (~30%) ganglionic eminences (MGE and CGE) and the preoptic area, embryonic germinal zones in the ventral telencephalon. We have recently demonstrated that, although the majority of neuronal populations (pyramidal neurons and MGE-derived interneurons) integrate into the cortex in an inside-out manner based on their birthdate, CGE-derived interneurons selectively integrate into the superficial layers irrespective of their birthdate (Miyoshi et al., 2010, Miyoshi and Fishell 2011). We have also shown that CGE-derive interneurons consist of a characteristic assemblage of VIP-expressing bipolar or bitufted classes and RELN-expressing neurogliaform or dense plexus cells that are distinct from MGE-derived

populations.
In order to understand the genetic programs controlling the development of CGE-derived interneurons, we undertook a microarray expression screen analysis. We found that the homeodomain gene Prox1 is selectively expressed within CGE-derived interneuron precursors at the time they tangentially migrate within the cortex. Moreover, Prox1 expression is maintained throughout adulthood within CGE-derived populations. By using conditional genetic strategies, we demonstrate that Prox1 is essential for both embryonic tangential migration and postnatal maturation of CGE-derived interneurons.

Disclosures: G. Miyoshi: None. **A. Young:** None. **T. Karayannis:** None. **M. McKenzie Chang:** None. **T. Petros:** None. **A. Lavado:** None. **T. Iwano:** None. **H. Taniguchi:** None. **M. Nakajima:** None. **J.Z. Huang:** None. **N. Heintz:** None. **F. Matsuzaki:** None. **G. Oliver:** None. **R. Machold:** None. **G. Fishell:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.04/A16

Topic: A.02. Neurogenesis and Gliogenesis

Title: Erbb4 gene expression is required for normal cochlear nucleus morphology

Authors: *K. T. YEE;
Neurobio. and Anatom. Sci., Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: The cochlear nucleus (CN), as the first, and only direct, CNS synaptic locus to receive sound information from the cochlea, is a critical brain region for auditory processing. Elegant anatomical and physiological studies have provided detailed characterization of the morphology and response properties of cochlear nucleus neurons. Investigations of the molecular control of the auditory system has focused on stages of functional hearing, its onset as well as its decline and less emphasis has been placed on the roles of molecules during CN development. However, more recent studies have begun to examine the effects of early molecular control. The laboratory is interested in how early gene expression affects CN morphology as a model system for neuronal development.
The receptor tyrosine kinase, Erbb4, is important for developmental events in the CNS, including neuronal migration and differentiation. The laboratory has previously reported that *Erbb4* has a perinatal expression profile in the CN. Both the dorsal and ventral subdomains of the CN express *Erbb4* at E16.5. At birth, *Erbb4* mRNA is localized to the molecular and granule cell layers and

in cells within the deep regions of the dorsal and ventral regions of the nucleus. Reported here is that in Erbb4 loss-of-function mutant mice, general histological staining shows perturbed dorsal CN organization including altered organization of the core region of the ventral CN. More detailed examination labeling auditory nerve fiber terminals (Zhou et al., 2007) in this region with an antibody against vesicular glutamate transporter 1 (vGlut1; Millipore) reveals altered organization of the ventral (V) CN core. Volume measurements delineated by vGlut1 localization show no difference between all major CN subdomains with the exception of the VCN core. The homozygous null VCN core shows expansion compared to wild type VCN (t-test. P=0.0032).
These data show that developmental expression of the receptor tyrosine kinase *Erbb4* contributes to the normal anatomical organization of the CN. The difference in pattern of vGlut1 immunohistochemical localization in the VCN between *Erbb4* null and wild type mice suggests that Erbb4 may be important for structural organization in the VCN, including the positioning of afferents on glutamatergic neurons. Studies suggest that cells in the VCN core may be involved in rapid processing of monaural acoustic spectral patterns required for speech or detection of other natural sounds (Oertel et al. 2000); a role for *Erbb4* in development of the VCN also supports its contribution to these auditory processes.

Disclosures: K.T. Yee: None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.05/A17

Topic: A.02. Neurogenesis and Gliogenesis

Support: FAPESP 2011/14329-9

Title: Length extension in FMRP KH-2 domain variable loop and neuronal differentiation

Authors: *L. A. HADDAD[1], F. J. VELLOSO[1], A. B. SAONA-MARÍN[1], J. C. CORREA[1], T. T. S. D. GOMES[2], H. ULRICH[3], L. R. G. BRITTO[4];
[1]Genet. and Evolutionary Biol., Univ. Sao Paulo, Sao Paulo, Brazil; [2]Cell and Developmental Biol., [3]Biochem., [4]Physiol. and Biophysics, Univ. de São Paulo, São Paulo, Brazil

Abstract: Fragile Mental Retardation Protein (FMRP) is necessary for synapsis maturation and elimination in the mammalian cerebral cortex, hippocampus and cerebellum. It is encoded by the Fragile Mental Retardation 1 (FMR1) gene and displays three types of RNA-binding domains: two Agenet motifs, two K-homology (KH) domains and one RGG box. FMRP second KH

domain (KH-2) has been implicated in RNA-binding specifically to kissing complex structures in vitro, and ribosome association in vitro and in vivo. KH-2 domain in FMRP contains a long variable loop that may be submitted to further extension after exon-12 in-frame inclusion in Fmr1 mRNA. Its extended form is significantly expressed in the cerebral cortex of the rat in a critical period for synaptogenesis (postnatal day (P) zero through P42). Here, we present immunohistochemistry data of P12 encephalon with two antibodies that specifically discriminate between FMRP isoforms expressing Fmr1 exon 12 (FMRP+12ISO) or total FMRP. Consistent with Western blotting results, we disclose a significant expression of those isoforms in P12 cerebral cortex pyramidal neurons from frontal (including prefrontal cortex), temporal and occipital lobes, and cerebellum Purkinje and granule cells, but not in hippocampus, where total FMRP was observed mostly in the dentate gyrus. Conversely, the histological analysis of the telencephalon between embryonic days (E) 12 and 20 revealed low expression of FMRP+12ISO in the neuroepithelium of the paired telencephalic vesicles (E12 - E14). On the other hand, those isoforms distribute to the cortical plate on E20 though less intensely than total FMRP. In neurospheres primarily cultured in suspension from E14 rat telencephalic vesicle, FMRP+12ISO were barely identified. Their expression was increased upon in vitro neurosphere neuronal differentiation. Altogether, our data confirm the highest expression of FMRP+12ISO in cerebral cortex neurons within the first 30 days of the rat postnatal period, and indicate that Fmr1 exon-12 expression directly relates to neuronal differentiation in vivo and in vitro. The specific functional roles of FMRP+12ISO in the cerebral cortex of the rat remain to be determined in the critical postnatal developmental period when synapses are maturing.

Disclosures: L.A. Haddad: None. **F.J. Velloso:** None. **A.B. Saona-Marín:** None. **J.C. Correa:** None. **T.T.S.D. Gomes:** None. **H. Ulrich:** None. **L.R.G. Britto:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.06/A18

Topic: A.02. Neurogenesis and Gliogenesis

Title: FoxD4 and neural development

Authors: *J. H. SHERMAN, M. FRALISH, B. KARPINSKI-OAKLEY, T. M. MAYNARD, S. A. MOODY, A. S. LAMANTIA;
The George Washington Univ., Washington, DC

Abstract: We evaluated the role of FoxD4, a forkhead family transcription factor, in the early steps of acquiring neural stem cell identity. In *Xenopus* embryos, FoxD4L1 serves as a key upstream regulator of numerous neural transcription factors and directs embryonic ectoderm towards a neural stem cell (NSC) lineage (Yan et al., 2009). The homologous gene in mice, FoxD4, is expressed in the embryonic central nervous system in a pattern similar to *Xenopus*. However, it is not known whether this transcription factor plays a similar role in promoting the development of NSCs in mammalian cells. We used a mouse ES to neural stem cell 7-day differentiation protocol to generate embryoid bodies (EB) and adherent neural stem cell cultures. FoxD4 expression appears after a neural differentiation step (RA treatment), which also causes a decline in pleuripotency markers (Nanog, Oct4, FoxD3). The peak expression of FoxD4 is concurrent with the expression of other markers of initial neural stem cell identity (Sox1, Isl1) and then declines as later neural progenitor (Nestin, N-Cad) and neuronal differentiation markers (neuronal beta-tubulin, neurofilament) emerge.. This time course of FoxD4 expression suggests that it is expressed in register with the transition between pleuripotent ES cells and initially committed neural stem cells. To evaluate the role of FoxD4 in this transition, we generated stable mESC lines that express siRNA "knockdown" constructs to diminish expression of *FoxD4*. We assessed differentiation of these "FoxD4-si" cells using the EBs for immature stem cell, neuronal precursor, and mature neuronal markers. We found that FoxD4-si cells retain ESC pluripotency. In early FoxD4-si EBs, undifferentiated cell markers (Oct4, FoxD3) remain expressed at high levels. In contrast, FoxD4-si derived EBs have decreased levels of neural differentiation markers (Zic1, N-cad) at slightly later stages in the differentiation protocol. Finally, FoxD4-si cells do not robustly express mature neuronal markers such as neurofilament and NCAM in adherent cultures that permit process outgrowth, nor is there extensive neurite growth from neurons derived from FoxD4-si cells. Together our results indicate that mammalian FoxD4 serves a parallel function to its *Xenopus* homologue as a key regulator of the transition from pluripotent ES cell to committed neural stem cell.

Disclosures: J.H. Sherman: None. **M. Fralish:** None. **B. Karpinski-Oakley:** None. **T.M. Maynard:** None. **S.A. Moody:** None. **A.S. LaMantia:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.07/A19

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant R01HD042182

Title: TxnRd2 imbalance during critical stages in development results in changes in mitochondrial distribution and neurite outgrowth

Authors: *A. FERNANDEZ[1,2,3,4], T. MAYNARD[1,3,4], D. MEECHAN[1,3,4], B. OAKLEY[1,3,4], A. LAMANTIA[1,3,4];
[2]GW Inst. of Biomed. Sci., [3]Pharmacol. and Physiol., [4]George Washington Inst. for Neurosci., [1]The George Washington Univ., Washington, DC

Abstract: Mitochondrial redox imbalance and decreased antioxidant defense may accompany altered cortical circuit organization and function in patients with psychiatric disorders. Patients with 22q11 deletion syndrome (22q11DS) are at increased risk for these disorders; therefore, 22q11DS provides a model for studying their neurodevelopmental origins—including the role of aberrant mitochondrial function. About one quarter of the genes located in the 22q11DS minimal deleted region encode proteins that localize to mitochondria. Among these, mitochondrial thioredoxin reductase (TxnRd2) encodes a primary mitochondrial H_2O_2 scavenger, and is maximally expressed during cortical circuit differentiation. We have asked whether changes in dosage of TxnRd2 compromise neuronal differentiation. We hypothesized that diminished mitochondrial antioxidant defense due to TxnRd2 imbalance during late stages of neuronal development would result in aberrant neurite outgrowth. Primary cortical neuronal cultures from E16.5 mouse embryos were electroporated with farnesylated-GPF and mitochondrial-flagged TxnRd2 knock-down and overexpression plasmids. TxnRd2 depleted neurons were also treated with ROS scavengers to reestablish intracellular redox balance and link TxnRd2 scavenging activity to normal neurite outgrowth. Changes in neurite outgrowth and mitochondrial distribution were evaluated by 3D reconstruction and Sholl analysis. Depletion of TxnRd2 results in decreased neurite outgrowth and complexity, while overexpression leads to no significant changes. Treatment of TxnRd2 depleted neurons with ROS scavengers returned neurite outgrowth to control levels. Overexpression of TxnRd2 leads to abnormal mitochondrial morphology and distribution. Changes in neurite outgrowth and mitochondrial morphology upon TxnRd2 imbalance suggest a key role of TxnRd2 in neuronal differentiation. Reestablishment of processes outgrowth in TxnRd2 depleted neurons treated with ROS scavengers implies an inverse correlation between ROS accumulation and TxnRd2 activity. These data suggests antioxidant defense as key in neuronal functional capacity and provides insight into the role of redox imbalance in abnormal circuit formation in a wide range of behavioral disorders.

Disclosures: A. Fernandez: None. **T. Maynard:** None. **D. Meechan:** None. **B. Oakley:** None. **A. LaMantia:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.08/A20

Topic: A.02. Neurogenesis and Gliogenesis

Support: Whitehall Foundation Grant

National Eye Institute Grant (EY020545)

Research to Prevent Blindness Grant

Title: Specificity of proneural basic helix-loop-helix transcription factors in retinal development

Authors: *T. J. KACZYNSKI[1,2,3], R. RUONALA[1,3], X. MU[1,2,4,3,5];
[2]Neurosci. Grad. Program, [3]Ctr. of Excellence in Bioinformatics and Life Sci., [4]Dept. of Ophthalmology/Ross Eye Inst., [5]SUNY Eye Inst., [1]SUNY Buffalo, Buffalo, NY

Abstract: Due to its accessibility and easy manipulation, the mammalian retina provides an ideal system for the study of neurodevelopment. Seven primary retinal cell types are produced during embryogenesis: rod and cone photoreceptors, horizontal, amacrine, and bipolar interneurons, retinal ganglion cells (RGCs), and the Müller glia. These cell types arise from a common pool of multipotent retinal progenitor cells (RPCs)
which differentiate into the various cell types primarily in response to the cell-autonomous inputs from transcription factors. The proneural transcription factors, which possess the basic helix-loop-helix (bHLH) DNA binding and dimerization motif, are critically involved in the genesis of retinal cell types from RPCs. All proneural factors possess a bHLH domain whose amino acid sequences are very similar to one another, and although each factor can bind identical DNA motifs, each plays a unique role in retinal development. The mechanism for the specificities of these transcription factors remains unknown.
For example, Math5 (also known as Atoh7) of this family is required for the production of RGCs, but another member, NeuroD, is required for amacrine cells and photoreceptors. To better understand which regions of Math5 are responsible for this specificity of function in the context of the retina, we performed domain-swap experiments between Math5 and NeuroD. Domain-swapped DNA constructs
were made possessing all or a portion of the bHLH region of one protein with the remaining flanking regions of the other. Math5 null mouse embryos, which lack the majority of RGCs, were electroporated with the constructs, and the retinas were examined for RGC formation. As expected, full-length Math5 could readily rescue RGC formation. In addition, the constructs containing the Math5 bHLH domain also rescued RGC formation, while constructs with the NeuroD bHLH domain failed to rescue RGC production even in cases where the flanking regions of Math5 were present. These results suggest that the bHLH domain of
the Math5 is a critical determinant of its specific function. More experimentation and analysis

are underway to further dissect the mechanism underlying the specificity of Math5 for the RGC fate.

Disclosures: T.J. Kaczynski: None. **R. Ruonala:** None. **X. Mu:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.09/A21

Topic: A.02. Neurogenesis and Gliogenesis

Support: Emmy Noether Program

SFB629

Kompetenznetzwerk Stammzellforschung NRW

EU FP7 network EuroSyStem

Münster Graduate Program CEDAD

Title: TRIM32-dependent microRNA activation is necessary for differentiation of neural stem cells

Authors: *S. NICKLAS[1,2], A.-L. HILLJE[1,2], I. MENZL[2], J. C. SCHWAMBORN[1,2]; [1]Luxembourg Ctr. For Systems Biomedicine, Esch-Belval, Luxembourg; [2]ZMBE, Inst. of Cell Biol., Münster, Germany

Abstract: During mouse embryonic brain development, neural stem cells undergo asymmetric cell divisions giving rise to one cell that maintains progenitor fate and one cell that differentiates into a neuron. Previously, we have shown that the cell fate determinant TRIM32 segregates asymmetrically to only one of these two daughter cells, where it suppresses self-renewal and induces neuronal differentiation by two mechanisms. On the one hand, the ubiquitin ligase TRIM32 ubiquitinates the transcription factor c-Myc, thereby targeting the protein for proteasomal degradation and inducing cell-cycle exit. Additionally, TRIM32 binds to the Argonaute-1 protein, which is part of the RNA-induced silencing complex. This interaction results in increased efficiency of post-transcriptional gene silencing through certain microRNAs. One of these microRNAs activated by TRIM32 is the stem cell regulator Let-7a, which is required and sufficient for neuronal differentiation. Furthermore, Let-7a has been shown to target c-Myc and to be in turn repressed by c-Myc. However, the exact mechanism of microRNA-

regulation by TRIM32 during neuronal differentiation has not been elucidated so far. Therefore, we are aiming at identifying functionally important components (protein and RNA) of the TRIM32/Argonaute complex that are important for the initiation of neuronal differentiation. In order to identify the protein components of this complex in neural stem cells we used a mass spectrometry approach. We were able to identify several new interaction partners which we analyzed in more detail with respect to their localization pattern in neural stem cells and their effect on microRNA activity in the presence and absence of TRIM32. Furthermore, via sequencing of TRIM32/Argonaute associated RNA, we aim at identifying microRNAs and mRNAs that are regulated by this complex. Finally, we want to analyze, how the newly identified protein components of the TRIM32/Argonaute complex regulate the activity of these associated mRNAs in a microRNA-dependent way. By this means we aim to unravel the molecular mechanism by which TRIM32 activates microRNAs to initiate neuronal differentiation in neural stem cells.

Disclosures: S. Nicklas: None. **A. Hillje:** None. **I. Menzl:** None. **J.C. Schwamborn:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.10/A22

Topic: A.02. Neurogenesis and Gliogenesis

Support: Human Frontier Science Program

Title: Dissecting a spatio-temporal Rho GTPase signaling network regulating neuronal growth cone extension

Authors: *G. AZARIAS[1], M. BAGONIS[2], L. FUSCO[1], G. DANUSER[2], O. PERTZ[1];
[1]Dept. of Biomedicin, Basel, Switzerland; [2]Dept. of Cell Biol., Harvard Medical School, MA

Abstract: Neuronal outgrowth determines the brain connectivity and requires tight spatio-temporal signaling to the cytoskeleton through the Rho family GTPases. Classical biochemical and cell biological studies have suggested that RhoA activates acto-myosin contractility, whereas Rac1 And Cdc42 allow neurite extension. However, recent experiments using FRET-based biosensors have indicated that Rho GTPase activation occurs in transient micrometer-sized subcellular domains, that correlate with cell edge morphological dynamics on sub-minute time scales.
Using real-time fluorescence imaging of FRET-based Rho GTPase biosensors in intact living cells, we quantified the spatio-temporal Rho GTPase activation dynamics within neuronal

growth cones at micrometer length and second time scale. Contrary to the classical dogma, we observed that all three canonical Rho GTPases are activated in the advancing growth cone in specific micrometer-sized subcellular zones. RhoA was activated at the tip of filopodia, Cdc42 was activated throughout the filopodium, and Rac1 was activated in the growth cone body and at the basis of filopodia. Thus, all three canonical Rho GTPases might collaborate to fine tune growth cone cytoskeletal dynamics by placing specific effector pathways in time and space. To address this issue, we developed a novel computer vision approach that allows automated filopodium dynamics segmentation for quantitative analysis of our dynamic timelapse datasets. This will be used to measure how the activation of the three GTPases fluctuate during the filopodium protrusion and retraction cycles and produce a multiplexed model of the activation dynamics of the three Rho GTPases.
Thus, our multidisciplinary methodology will allow resolving a complex spatio-temporal signaling network at the time and length scale on which growth cone cytoskeleton operates.

Disclosures: G. Azarias: None. **M. Bagonis:** None. **G. Danuser:** None. **L. Fusco:** None. **O. Pertz:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.11/A23

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant NS029169

Title: Osteopontin is expressed by the largest retinal ganglion cells (alpha RGCs) and causes RGC enlargement

Authors: *M. QIAO[1], X. DUAN[2], J. R. SANES[2];
[1]MCB, [2]Harvard Univ., Cambridge, MA

Abstract: The mammalian retina contains >20 different retinal ganglion cell (RGC) subtypes, each with distinct molecular, morphological and physiological properties. The largest are called alpha RGCs (aRGCs); in mice, Pang et al. (J. Neurosci, 2003) described three aRGCs subtypes, differing in dendritic morphology and visual responses. In the course of developing reagents to mark and manipulate retinal subtypes, we generated a mouse line in which Cre recombinase is inserted into the KCNG4 locus. This line marks subsets of RGCs with large somas and dendritic fields, as well as some bipolar cells. Labeled RGCs were present throughout the retina and were neurofilament-positive, suggesting they were aRGCs. Single cell labeling showed that the

KCNG-positive RGCs comprise three subtypes with distinct dendritic laminar restrictions. Physiological analysis followed by dye-filling revealed that these three subtypes correspond to the ON sustained, OFF sustained and OFF transient aRGCs described by Pang et al. Furthermore, analysis of double-transgenic mice demonstrated that the KCNG4 RGCs include all RGCs labeled in the CB2-GFP and W7-YFP lines, previously shown to label OFF aRGC subtypes (Huberman et al. 2008; Kim et al. 2010). Thus, the RGCs labeled in the KCNG4 line include nearly all and nearly only aRGCs.
We then used these lines for molecular analysis, and found that all aRGCs labeled in all three lines (KCNG4, CB2 and W7) express an O-glycosylated phosphoprotein, osteopontin (OPN), and that all OPN-positive RGCs are aRGCs. Developmental analysis shows that expression of OPN increases during the postnatal interval when somata of aRGCs become larger than those of other RGCs. Knowing that osteopontin regulates growth in nonneuronal cells (Rangaswami et al. 2006), we have begun to test the idea that OPN promotes RGC growth. We used an adeno-associated virus (AAV) to express OPN in non-aRGCs, and found that it leads to an increase in their soma size. These results suggests the possibility that OPN contributes to making aRGCs the largest RGCs. (Supported by NIH.)

Disclosures: M. Qiao: None. **X. Duan:** None. **J.R. Sanes:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.12/A24

Topic: A.02. Neurogenesis and Gliogenesis

Support: Center for Behavioural Brain Sciences

German Research Foundation SFB854 TP10

German National Academic Foundation

German Research Foundation GRK1167

Title: The ubiquitin ligase Praja1 reduces NRAGE expression and inhibits neuronal differentiation of PC12 cells

Authors: *J. TEUBER[1], B. MUELLER[1,2], R. FUKABORI[1], D. LANG[1], A. ALBRECHT[1], O. STORK[1];

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Abstract: Evidence suggests that regulated ubiquitination of proteins plays a critical role in the development and plasticity of the central nervous system. We have previously identified the ubiquitin ligase Praja1 as a gene product induced during fear memory consolidation. However, the neuronal function of this enzyme still needs to be clarified. Hence, we investigated its involvement in the nerve growth factor (NGF)-induced differentiation of rat pheochromocytoma (PC12) cells. Praja1 has been shown to precipitate the neurotrophin receptor interacting MAGE homologue (NRAGE) in a GST pull-down and to occur in a complex with NRAGE and Msx2. NRAGE is a mediator of p75NTR-dependent NGF signalling and has been identified as a potent modulator of apoptosis. Aside from this, contradictory effects on neurite outgrowth in PC12 have been described. Our findings show that Praja1 co-localizes with cytoskeleton components and decreases the pro-apoptotic effects of NRAGE. Furthermore, we observed an enhanced expression of Praja1 after 3 days of NGF treatment and a suppression of neurite formation upon Praja1 overexpression in stably transfected PC12 cell lines, which was associated with a proteasome-dependent reduction of NRAGE levels. Our data thus suggest that Praja1, through ubiquitination and degradation of NRAGE, inhibits neuronal differentiation. The two murine isoforms, Praja1.1 and Praja1.2, appear to be functionally homologous in this respect.

Disclosures: J. Teuber: None. **B. Mueller:** None. **R. Fukabori:** None. **D. Lang:** None. **A. Albrecht:** None. **O. Stork:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.13/A25

Topic: A.02. Neurogenesis and Gliogenesis

Title: MicroRNAs in neurite outgrowth and maturation of midbrain neurons

Authors: *A.-E. ROSER[1,2,3], L. TÖNGES[1], R. HALDER[4], J. DYCZKOWSKI[4], M. BÄHR[1,2], A. FISCHER[2,4], P. LINGOR[1,2];
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Abstract: miRNAs are small non-coding RNAs that are important in post-transcriptional regulation of gene expression. The functions of miRNAs in neurons are just beginning to emerge, however there are indications that miRNAs drive neuronal differentiation and morphogenesis through specific expression patterns. Understanding these changes in the miRNAome during neurite outgrowth would lead to new insights into the molecular processes involved and might provide new therapeutical targets to treat diseases of the central nervous system. We employed massive parallel sequencing and present the complete and quantitative miRNAome of murine primary midbrain neurons at different time points of neuronal maturation and neurite outgrowth. 848 out of 1410 murine miRNAs were detected. Differential expression analysis revealed that the major changes in miRNA expression occur between day 1 and day 5 in vitro. 163 miRNAs were significantly regulated and 47 of these showed a more than 4-fold change in expression levels. Target prediction and functional annotation of these predicted target genes were performed to identify miRNAs involved in neurite outgrowth. As the midbrain is central target of neurodegenerative diseases such as Parkinson's disease and has limited regenerative capacities, detailed knowledge about miRNA-mediated regulation of molecular processes involved in neurite outgrowth may be useful for the identification of future therapeutic targets in degenerative CNS diseases.

Disclosures: A. Roser: None. **L. Tönges:** None. **R. Halder:** None. **J. Dyczkowski:** None. **M. Bähr:** None. **A. Fischer:** None. **P. Lingor:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.14/A26

Topic: A.02. Neurogenesis and Gliogenesis

Support: Münster Graduate Program for Cell Dynamics and Disease (CEDAD)

German Research Foundation (DFG: Emmy Noether Program)

Kompetenznetzwerk Stammzellforschung NRW

SFB629

EU FP7 Network Eurosystem

Title: TRIM32 dependent transcription in adult neural progenitors regulates neuronal differentiation and olfactory learning

Authors: *M. A. PAVLOU[1,2], A.-L. HILLJE[1,2], E. BECKMANN[3], M. WORLITZER[2], L. BAHNASSAWY[1], L. LEWEJOHANN[4], T. PALM[2], J. SCHWAMBORN[1,2];
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Abstract: In the adult brain, neurogenesis is restricted to the subventricular zone (SVZ) of the lateral ventricles and the dentate gyrus of the hippocampus. Adult neural stem cells in the subventricular zone continuously generate new neurons for the olfactory bulb. As a defining feature of all stem cells, also these adult NSCs have the ability to simultaneously self-renew and generate more fate committed daughter cells. Astrocytes (also known as type B cells) localized in the SVZ, function as primary progenitors that give rise to fast dividing transit amplifying cells (type C cells) which then differentiate into neuroblasts (type A cells). Neuroblasts leave the SVZ and migrate through the rostral migratory stream (RMS) to their final destination the olfactory bulb, where they differentiate into neurons. This fate commitment is regulated by cell fate determining proteins. The TRIM-NHL protein family has an evolutionary conserved function in neuronal cell fate specification. In mammals the protein TRIM32 is so far the only asymmetrically segregating neuronal fate inducing determinant. Here, we show that the cell fate determinant TRIM32 is upregulated during maturation of adult neural stem cells into olfactory bulb neurons. This upregulation is accompanied by neural progenitor cell divisions which show an asymmetric distribution of TRIM32 during mitosis. We further demonstrate that TRIM32 is necessary and sufficient for neuronal differentiation in adult neural stem cells, through overexpression and knockdown experiments in vivo using stereotactic injections. Interestingly, TRIM32-deficency induced overproduction of new olfactory bulb neurons, leads to a clear impairment in olfactory learning processes. Thus, we provide evidences for a function of TRIM32 for neuronal differentiation of adult neuroblasts and we correlate the cell fate determinant dependent activity of adult neurogenesis with complex learning behavior.

Disclosures: M.A. Pavlou: None. **A. Hillje:** None. **E. Beckmann:** None. **M. Worlitzer:** None. **L. Bahnassawy:** None. **L. Lewejohann:** None. **T. Palm:** None. **J. Schwamborn:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.15/B1

Topic: A.02. Neurogenesis and Gliogenesis

Title: Contributions of bHLH transcription factors to dopaminergic and neuroendocrine differentiation in the zebrafish ventral diencephalic Orthopedia expressing precursor domain

Authors: *M. RATH, W. DRIEVER;
Developmental Biol., Univ. of Freiburg, Freiburg, Germany

Abstract: During neural development, patterning mechanisms specify regional identities in a coordinated fashion in the vertebrate brain. Expression domains of specific transcription factors then drive differentiation of one or several different neuronal identities in these anatomical domains. The Orthopedia (Otp) transcription factor defines a domain within the ventral diencephalon, including preoptic and hypothalamic territories, where dopaminergic A11-type neurons as well as neuroendocrine and other neuronal cell types develop. How specific neuronal identities are established within the Otp expressing regions is not well understood. In principle, more refined regional patterning mechanisms, differentially migrating precursor populations, but also proneural transcription factors driving lineage decisions, may contribute to neuronal cell type diversity.
We use the zebrafish model to investigate dopaminergic (DA) and neuroendocrine neuron differentiation within the Otp-expressing precursor region. In this region, next to DA, corticotrophin-releasing hormone (CRH), isotocin (ITNP) and vasotocin (VSNP) neurons form. The transcription factors Otp, Aryl-hydrocarbon receptor nuclear translocator 2 (Arnt2), and Single-minded 1 (Sim1) act together during specification of these neurons (Ryu et al. 2007, Löhr et al. 2009). Loss-of-function for each of these transcription factors reduces both DA and neurosecretory cell types. The mechanisms downstream of Otp that segregate DA from the other lineages are currently unknown. Given that proneural bHLH transcription factors have previously been shown to mediate other lineage decisions in the hindbrain and spinal cord, we investigated whether the proneural bHLH factors Ngn1, Olig2, or Ascl1a, which have previously been shown to be involved in DA development (Jeong et al., 2006; Brodowsky et al., 2009; Liu et al., 2011), may contribute to lineage decisions in the Otp domain. We also included in our analysis additional bHLH factors identified by transcriptional profiling. Further, DA neurons, but also CRH neurons, of the hypothalamus are characterized by double neurotransmitter phenotypes, which we analyze to determine shared and unique features of transcriptional control of the complex neurotransmitter identity of these neurons.

Disclosures: M. Rath: None. **W. Driever:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.16/B2

Topic: A.02. Neurogenesis and Gliogenesis

Support: The Japan Society for the Promotion of Science (JSPS)

Title: Maintenance DNA methyltransferase DNMT1 controls neuronal differentiation of late-gestational neural stem cells

Authors: *H. NOGUCHI[1,2], M. NAMIHIRA[4], T. SANOSAKA[1], K. TSUJIMURA[1], Y. FUKAO[3], K. IGARASHI[5], A. KIMURA[1], K. NAKASHIMA[1];
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Abstract: DNA methylation is known to influence a variety of biological aspects such as development, cell differentiation and genomic reprogramming. In mammals, DNA methylation patterns are established and maintained by DNA methyltransferases, DNMT1, -3A, and -3B. During cortical development, neurons and glial cells (astrocytes and oligodendrocytes) are generated from common multipotent neural stem cells (NSCs) in a temporal order, in which neurons are generated first at mid-gestation, followed by glial cells at late-gestation. During mid-gestation, when neuronal differentiation is dominant, DNMT1 plays an essential role in the inhibition of precocious astrocyte differentiation by maintaining DNA methylation of astrocytic gene promoters. Previously, we observed that DNMT1 expression was not only confined to mid-gestation, but expressed by NSCs in all development stages. The prevalence of DNMT1 expression suggests a novel role of DNMT1 regulating cortical development. Toward the functional analysis of DNMT1 in late-gestational neural stem cell (lg-NSC), we found that NSCs overexpresing DNMT1 were stalled at VZ/SVZ, instead of following the differentiation and migration route. Targeted DNMT1 knock out in lg-NSC led to increased cell cycle exit, resulting in diminished stem cell pool. Furthermore, shRNA-mediated DNMT1 knock down in cultured lg-NSCs resulted in increased neuronal differentiation, while the overexpression of DNMT1 inhibited neuronal differentiation of lg-NSCs. Interestingly, overexpression of mutant DNMT1 lacking methylation activity also reduced neuronal differentiation of lg-NSCs. These results suggest DNMT1 as a neuronal differentiation repressor of NSCs during late-gestation and this repression occurs in a DNA methylation-independent manner. To explore the molecular mechanisms, we will be seeking to identify the target genes and interacting proteins of DNMT1 *via* the gene expression profiling and proteomic analysis, respectively.

Disclosures: H. Noguchi: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The Japan Society for the Promotion of Science (JSPS). **M. Namihira:** None. **T. Sanosaka:** None. **K.**

Tsujimura: None. **Y. Fukao:** None. **K. Igarashi:** None. **A. Kimura:** None. **K. Nakashima:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.17/B3

Topic: A.02. Neurogenesis and Gliogenesis

Support: NHRI-EX102-9919NC

Title: The role of N-cadherin cytoplasmic terminal fragment in modulating beta-catenin activity during brain development

Authors: *M.-H. WEN[1,2], M.-P. WANG[1], C.-Y. TAI[1];
[1]Inst. of Mol. Biology, Academia Sinica, Nankang, Taipei, Taiwan; [2]Grad. Inst. of Life Science, Natl. Def. Med. Ctr., Taipei, Taiwan

Abstract:
N-cadherin works together with its intracellular binding partner beta-catenin in neuronal migration, lamination and synaptic connections. On the other hand, beta-catenin is also a key mediator for Wnt signaling, which drives the proliferation of neuroprogenitor cells in early brain development. However, the role of N-cadherin in this Wnt/beta-catenin mediated event remains unclear. Recent evidence has demonstrated that the cytosolic-terminal fragment (CTF) of N-cadherin can be released from membrane upon gamma-secretase mediated proteolysis. Therefore we speculate that the released N-cadherin CTF may modulate Wnt/beta-catenin functions. Here we show that the protein level of N-cadherin CTF is higher in embryonic than in adult brains. This developmental switch of N-cadherin CTF level correlates with the gamma-secretase activity. Overexpression of CTF facilitates nuclear translocation of beta-catenin both in heterologous cells and cultured neurons. However the transcriptional activity of beta-catenin is suppressed, despite the elevated protein level inside the nucleus. Together, our results suggest that N-cadherin may negatively regulate Wnt/beta-catenin signaling via proteolytic regulation during brain development.

Disclosures: M. Wen: None. **M. Wang:** None. **C. Tai:** None.

Poster

510. **Neuronal Differentiation: Mechanisms I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 510.18/B4

Topic: B.11. Glia-Neuron Interactions

Title: Evidence for the involvement of DNA polymerase β in the regulation of neuronal energy metabolism

Authors: *M. M. MISIAK[1,2], P. SYKORA[1], D. CROTEAU[1], M. P. MATTSON[2], V. A. BOHR[1];
[1]Lab. of Mol. Gerontology, NIH/National Inst. On Aging, Baltimore, MD; [2]Lab. of Neurosciences, NIH/National Inst. on Aging, Baltimore, MD

Abstract: DNA repair is essential for brain development, and impaired DNA repair is implicated in developmental and adult neurological disorders. As the primary DNA polymerase involved in base excision repair (BER), DNA polymerase β (Pol β) is critical for preventing mutations, and is also required for proper neurogenesis during brain development. In the absence of Polβ, neurons in the brains of mice undergo massive perinatal apoptosis. However, the cause of this neuronal apoptosis is unknown. Interestingly, even when apoptosis is prevented by the concomitant deficiency of the pro-apoptotic factor p53, mice still display major brain abnormalities, suggesting that Polβ is required for normal neural progenitor cell differentiation. Additionally, astrocytes are important for maintaining and supporting neuronal functions and metabolism, such that when astrocytes are missing or their function is impaired, neurons cannot function properly and may die. Therefore, we postulate that neuronal apoptosis and embryonic death shortly after birth in Polß-/- mice might be related to impaired proliferation and/or differentiation of astrocytes, and to an associated metabolic impairment. To test this hypothesis, we cultured cortical cell neurospheres from wild type (WT) and Polβ-/- embryos and differentiated those progenitor cells into neurons or astrocytes. Polβ-/- neurons showed normal morphology and were able to grow neurites. Cultured Polβ-/- astrocytes grew significantly slower than WT astrocytes, suggesting a proliferation defect. Analysis of mitochondrial metabolism in neurons revealed that Polβ-/- neurons exhibit higher basal oxygen consumption, lower reserve capacity, and are unable to switch to glycolysis when oxidative phosphorylation is inhibited. This metabolic abnormality in Polβ-/- neurons may render them highly dependent on astrocytes for lactate production. We are currently performing studies aimed at establishing the molecular mechanisms by which deficiency in a DNA repair enzyme adversely affects neuronal energy metabolism and vulnerability to synaptic dysfunction and degeneration.

Disclosures: M.M. Misiak: A. Employment/Salary (full or part-time):; NIH/NIA/IRP. **P. Sykora:** A. Employment/Salary (full or part-time):; NIH/NIA/IRP. **D. Croteau:** A. Employment/Salary (full or part-time):; NIH/NIA/IRP. **M.P. Mattson:** A. Employment/Salary

(full or part-time):; NIH/NIA/IRP. **V.A. Bohr:** A. Employment/Salary (full or part-time):; NIH/NIA/IRP.**Poster**

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.01/B5

Topic: A.02. Neurogenesis and Gliogenesis

Support: ALLEN INSTITUTE for BRAIN SCIENCE48JE0059IN 541A 68301

MICINN FONDOS FEDER REF. BFU2010-27326

Title: Molecular microdomains of germinative epithelium in mouse cerebellum generate different clusters of cerebellar cortical neurons

Authors: *R. GARCIA-LOPEZ[1], A. POMBERO[1], J. HOHMANN[2], C. THOMPSON[2], K. GLATTFELDER[2], V. MENON[2], W. WAKEMAN[2], M. HAWRYLYCZ[2], C. DANG[2], S. MARTINEZ[1];
[1]Inst. Neurociencias, San Juan De Alicante, Spain; [2]Allen Inst. for Brain Science,, Seattle, WA

Abstract: The cerebellum is an attractive brain region due to its sophisticated circuitry, high degree of modifiability combined with unique operational mechanisms, and the growing awareness of its multiple roles in animal motor and cognitive behavior. The mature cerebellum is a highly organized structure partitioned into lobules and lamella along the anterior-posterior (A-P) axis, and into striped molecular domains along the medial-lateral (M-L) axis. We have studied the expression patterns of 67 genes (expressed in E13.5 and E15.5 mouse cerebellar ventricular epithelium) at E15.5, E18.5 and postnatal (P4, P14, P28) stages using the Allen Developing Mouse Brain Atlas (www.brain-map.org/). Then we generated combinatorial maps of regional expression patterns in order to: 1) establish molecular references to detect morphogenetic field limits in developing cerebellum; 2) analyze maintenance of the ventricular molecular labeling in cellular migratory streams; 3) describe specific molecular markers for neuroepithelial presumptive regions and 4) identify potential regulatory genes underlying neural differentiation in cerebellar cortex. The quality and the resolution of the data revealed previously undetected molecular microdomains of germinative epithelium in mouse cerebellum that can be related to the complex origin of cerebellar plate, from mesencephalic, isthmic and rhombomere1 progenitors.

Disclosures: R. Garcia-Lopez: None. **A. Pombero:** None. **S. Martinez:** None. **J. Hohmann:** None. **C. Thompson:** None. **K. Glattfelder:** None. **V. Menon:** None. **W. Wakeman:** None. **M. Hawrylycz:** None. **C. Dang:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.02/B6

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grants

Title: Making the match: How are postsynaptic receptors paired with presynaptic transmitters?

Authors: *D. R. HAMMOND-WEINBERGER[1], N. C. SPITZER[1,2];
[1]Neurobio. Section, Div. of Biol. Sci., UCSD, La Jolla, CA; [2]Kavli Inst. for Brain and Mind, La Jolla, CA

Abstract: Electrical activity participates in the specification of neurotransmitters in different classes of neurons. At the developing Xenopus neuromuscular junction, motor neurons normally express the neurotransmitter acetylcholine (ACh) paired with cognate ACh receptors on muscle. In addition to ACh receptors, developing muscle cells express non-cholinergic receptors before innervation that are subsequently eliminated by an unknown mechanism. Changes in electrical activity of these immature motor neurons during a critical period specify a homeostatic shift to non-cholinergic neurons and the retention of matching, non-cholinergic receptors from the initially diverse receptor pool, identified anatomically and functionally. Enhanced activity results in a switch from excitatory ACh to inhibitory GABAergic or glycinergic neuronal phenotypes and retention of GABA or glycine receptors. Reduced activity results in a switch from ACh to combinations of cholinergic and glutamatergic phenotypes, with the retention of both ACh and glutamate receptors. The mechanism of receptor matching remains elusive.
We cultured neurons and muscle cells in the presence or absence of extracellular Ca2+ to alter neuronal activity and sustain or increase the number of glutamatergic neurons. Although the 0-Ca2+ medium failed to yield an immunocytochemically detectable increase in expression of ionotropic glutamate receptors on either innervated or uninnervated muscle cells, a greater number of cells were sensitive to exogenous glutamate, as assayed with the fluorescent Ca2+ indicator Fluo-4AM. The increase in glutamate sensitivity was blocked when NBQX and AP5 were added to the culture medium to block ionotropic glutamate receptors and washed out before testing glutamate sensitivity. Thus, changes in receptor function appear to precede changes in anatomically identified receptor populations, and receptor activation is necessary for the increase in sensitivity. We are determining whether receptor activation is also sufficient to increase the glutamate sensitivity in cultures of muscle cells without neurons and are testing the roles of Ca2+-dependent kinases in silencing the glutamatergic receptor population in developing muscle

cells. Activation of glutamate receptors may shift the balance of intracellular Ca2+ signaling and affect the maintenance of appropriate receptors and elimination of inappropriate classes as a homeostatic mechanism to maintain a functional circuit. This research is expected to be pertinent to understanding the dysregulation of transmitter receptors associated with neurological and psychiatric disorders. Supported by NIH grants.

Disclosures: D.R. Hammond-Weinberger: None. **N.C. Spitzer:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.03/B7

Topic: A.02. Neurogenesis and Gliogenesis

Support: Childhood Brain Tumor Foundation

American Cancer Society

Title: Regulation of p53 during neuronal differentiation

Authors: *S. GHASSEMIFAR, S. MENDRYSA;
Basic Med. sciences, Purdue Univ., West Lafayette, IN

Abstract: During nervous system development, failure of differentiation of neural stem cells (NSCs) and their derivative cells to mature neurons allow these cells to continue proliferating and is speculated to contribute to the development of embryonal brain tumors including medulloblastoma (MB), the most common malignant brain tumor in children. Although current treatments are capable of killing these tumors, they are also toxic to normal cells leading to devastating side effects especially in infants whose nervous system is still developing. A potentially less toxic approach to treat these tumors is to promote cell differentiation. The p53 tumor suppressor protein has been implicated in the regulation of neuronal differentiation in addition to having important roles in apoptosis, proliferation and self-renewal. Given the fact that p53 is not mutated in most embryonal brain tumors, development of strategies that specifically activates p53 prodifferentiation function is predicted to benefit the patients with these tumors. In this study, we investigated how p53 function is regulated during neuronal differentiation by employing the human embryonal teratocarcinoma NT2 cell line in which neuronal differentiation can be induced by retinoic acid treatment. We found that p53 is activated in differentiating NT2 cells concomitant with a decrease in level of MDM2, the key inhibitor of p53. Our data support a model in which proteasomal degradation of MDM2 is enhanced upon neuronal differentiation.

Consistent with our model, we observed a change in the levels of several regulators of MDM2 stability including USP2a, HAUSP and PCAF. Our findings identify MDM2 as a potential target for differentiation therapy of the embryonic brain tumors in which p53 is wild type.

Disclosures: S. Ghassemifar: None. **S. Mendrysa:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.04/B8

Topic: A.02. Neurogenesis and Gliogenesis

Support: CSIR

ICMR

DBT

RGCB intramural fund

Title: Regulation of retinal ganglion cell fate specification and differentiation by miR-23a and miR-374 during retinal development

Authors: *A. VAZHANTHODI[1], S. SREEKANTH[2], M. S. DIVYA[1], T. S. DIVYA[1], S. B. DHANESH[1], C. SUBASHINI[1], V. ANI DAS[2], J. JAMES[1];
[2]Mol. Neurobio. Lab., [1]RAJIV GANDHI CENTRE FOR BIOTECHNOLOGY, THIRUVANANTHAPURAM, India

Abstract: Retinal ganglion cells (RGCs) are one of the specialised neurons among the seven cell types in the retina that transmit the visual signals to the brain. The timing of RGC genesis, the first formed retinal cell type, from its progenitors and its maturation into a functional neuron is an important aspect that still remains as a piece of puzzle. The development of RGCs are controlled by various extrinsic factors such as Shh, FGF2, etc. and intrinsic regulators that include transcription factors such as Ath5, Brn3b, Isl1, Wt1, etc. Brn3b or Pou4f2, a POU domain binding transcription factor, has been found to play a major role in RGC survival and axonal guidance. The question that we asked next was regarding the factors that regulate Brn3b expression and how it regulates the RGC development. Recent studies have shown microRNAs to be involved in regulating the expression of various genes post transcriptionally thereby regulating their translation. For this we carried out *in silico* screening for microRNAs that target Brn3b and narrowed down to miR-23a and miR-374 for further analysis, since these two were

picked up by multiple programs. Temporal expression analyses of these miRNAs demonstrate that their expressions are well orchestrated to regulate the expression of Brn3b pattern during mouse retinal development. We further analyzed its role during retinal development and found that miR-23a and miR-374 by itself could not significantly regulate the expression of Brn3b but when expressed together could precisely down regulate Brn3b and also affect the patterning of the retina. Further we corroborated these finding in E14 retinal explants after *ex vivo* electroporation where we found altered patterning in Ganglion cell layer with loss of Brn3b expressing cells upon combined expression of miR-23a and miR-374. These finding highlight a post transcriptional regulatory mechanism of Brn3b, an intrinsic factor involved in RGC differentiation, survival and axonal growth during retinal development. In view of these finding we are currently pursuing the role of these miRNA in the maturation and axonal guidance of RGCs.

Disclosures: A. Vazhanthodi: None. **S. Sreekanth:** None. **M.S. Divya:** None. **T.S. Divya:** None. **S.B. Dhanesh:** None. **C. Subashini:** None. **V. Ani Das:** None. **J. James:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.05/B9

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH F31 NS070559

Title: Transcription factor network specifying inhibitory and excitatory neurons in the dorsal spinal cord

Authors: *M. D. BORROMEO[1], D. MEREDITH[1], D. S. CASTRO[2], K. C. TUNG[1], F. GUILLEMOT[3], J. E. JOHNSON[1];
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Abstract: The proper formation of the central nervous system (CNS) requires the proper balance of excitatory and inhibitory neurons; disruption of this balance may cause neurological disorders, such as somatosenory disorders and epilepsy. Neural basic helix-loop-helix (bHLH) transcription factors play a crucial role in generating the correct number and sub-types of neurons in the dorsal spinal cord. Little is known about the transcriptional targets of these regulators particularly as it relates to their neuronal subtype specification functions. We searched for potential direct downstream transcriptional targets of two neural bHLH factors Ascl1 and Ptf1a during the

neurogenic stage of the developing mouse spinal cord by using chromatin immunoprecipitation coupled to massive parallel sequencing (ChIP-Seq), and by coupling transcriptome analysis (mRNA-Seq) from isolated Ascl1 and Ptf1a lineage cells. We show Ascl1 and Ptf1a not only regulate genes important for neuronal differentiation, but also genes that are important for specification of the excitatory and inhibitory neurons in the dorsal spinal cord. Ascl1 directly activates a glutamatergic specification program that includes specific transcription factors Tlx1/3 and Lmx1b. In contrast, Ptf1a directly activates a GABAergic specification program that includes specific transcription factors Lhx1, Lhx5, and Pax2; and components of the GABA biosynthesis and transport pathway, such as Gad1 and Slc32a1. Current analyses are directed towards distinguishing how Ascl1 and Ptf1a select their specific targets in the developing spinal cord.

Disclosures: M.D. Borromeo: None. **D. Meredith:** None. **D.S. Castro:** None. **K.C. Tung:** None. **F. Guillemot:** None. **J.E. Johnson:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.06/B10

Topic: A.02. Neurogenesis and Gliogenesis

Title: Selective expression and developmental impact of the Met receptor tyrosine kinase in B6 subgroup of serotonin neurons

Authors: *H.-H. WU[1], S. CHOI[1], K. KIKUMA[2], E. DENERIS[3], P. LEVITT[1,2];
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Abstract: The autism risk gene, Met receptor tyrosine kinase, and its ligand, HGF have been implicated in a wide variety of roles during nervous system development. *Met* expression and its role in mouse forebrain development have been under extensive investigation. However, the expression patterns and function of *Met* in the developing brainstem is less well understood. In situ mapping of *Met* transcript revealed positive neurons in a highly restricted domain of the developing dorsal raphe (DR) up to birth. Immunocytochemical co-localization revealed that a subset of serotonergic raphe neurons is MET^+. Detailed mapping in serial coronal sections at embryonic day (E)16 and sagittal sections from E16 to postnatal day (P)4 revealed that MET is expressed almost exclusively in the caudal part of DR as a paired nucleus situated just below the aqueduct, a region corresponding to the B6 subgroup of the raphe. Double immunostaining on

sections from *Pet-1* knockout mice (*Pet-1KO*) was performed. Remarkably, there was no detectable MET protein expression in the DR of homozygous *Pet-1* null mice, even though residual 5-HT^{+} neurons were observed. These data suggest that MET expression in B6 subgroup of DR 5-HT neurons is Pet-1 dependent. Initial analyses of deleting *Met* with the *Pet-1*^{Cre} line suggest that MET signaling may be important for normal B6 neuron assembly. Finally, a bioinformatics analysis using several public databases generated a listing of B6-enriched genes. Gene expression of candidates is being evaluated in WT, *Pet-1KO*, and *Pet-1*^{Cre} x *Met*^{fx} mice.

Disclosures: H. Wu: None. **S. Choi:** None. **K. Kikuma:** None. **E. Deneris:** None. **P. Levitt:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.07/B11

Topic: A.02. Neurogenesis and Gliogenesis

Support: German Research Foundation Excellence Cluster NeuroCure (Exc 257)

Heisenberg Program

German Research Foundation Research Center of Molecular Physiology of the Brain

Title: Neurod1/2/6 regulate hippocampal pyramidal neuron differentiation and survival

Authors: *O. GRISHINA[1], I. BORMUTH[1], K. YAN[1], T. YONEMASU[2], S. GOEBBELS[2], K. A. NAVE[2], M. H. SCHWAB[2], V. TARABYKIN[1];
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Abstract: The hippocampus is probably the best studied part of the cerebral cortex. It is essential for learning and memory and accepted as model system for cortical information processing. The hippocampal formation is comprised of pyramidal neurons (cornu ammonis), granule cells (dentate gyrus), and interneurons. During embryonic development, neuroepithelial cells in the ventricular zone give rise to neuronal precursors which migrate and differentiate into functional granule and pyramidal neurons. The molecular mechanisms of these differentiation processes are still poorly understood.
Granule cell differentiation has been shown to dependent on the bHLH transcription factor Neurod1. In Neurod1 deficient mice, granule neurons undergo apoptosis resulting in agenesis of

the dentate gyrus. The generation of pyramidal neurons is not affected by inactivation of Neurod1 alone. These cells express two related factors, Neurod2 (NDRF) and Neurod6 (NEX), which serve redundant functionality.
We studied Neurod1/2/6 triple mutant mice and found hippocampal pyramidal neurons to stop differentiation and undergo apoptosis in a very similar manner. The mitotic activity in the ventricular zone of Neurod1/2/6 triple mutants is not reduced. Immature pyramidal neurons migrate radially and become apoptotic shortly after futile activation of the endogenous Neurod6 promoter. The generation of interneurons and glial cells is not affected in Neurod1/2/6 triple mutant mice. Intrestingly, pyramidal neurons can survive and differentiate in dissociated neuron cultures but not organotypic slice cultures prepared from Neurod1/2/6 mutant hippocampal tissue. This argues for a cell extrinsic effect and enables us to study the underlying mechanisms on a cellular level.

Disclosures: O. Grishina: None. **I. Bormuth:** None. **K. Yan:** None. **T. Yonemasu:** None. **S. Goebbels:** None. **K.A. Nave:** None. **M.H. Schwab:** None. **V. Tarabykin:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.08/B12

Topic: A.02. Neurogenesis and Gliogenesis

Support: West Michigan Science and Technology Innovation Grant

GVSU Student Summer Scholars and Research Grant-in-Aid

Title: Nato3 induces the expression of key DA neuron markers in a regionally and temporally specific manner within the developing CNS

Authors: J. L. STRAIGHT[1], D. PETERSON[1], *M. K. TAYLOR[2];
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Abstract: In the developing stages of the central nervous system (CNS), neural stem cells gradually adopt specific cell fates and differentiate accordingly. The floor plate of the developing midbrain gives rise to dopaminergic (DA) neurons, an important class of neurons involved in Parkinson's disease. Better understanding of the mechanisms by which DA neurons are created is of great interest and would accelerate promising applications such as cell replacement therapies. Nato3, a basic helix-loop-helix transcription factor, is expressed in the floor plate

region of the midbrain during development. In vitro studies suggest that Nato3 overexpression is sufficient to promote floor plate and DA neuron marker expression, whereas in vivo studies suggest that Nato3 is not. Here, we show that overexpression of Nato3 in the developing chick produces a regionally and temporally dependent increase in DA neuron markers Nurr1 (an immature DA neuron marker) and tyrosine hydroxylase (TH) (a mature DA neuron marker) within the ventral midbrain. In-ovo electroporation was used for transfection, and Nato3 overexpression was monitored using a bicistronic EGFP reporter expression vector. The observed effects were characterized by quantitative PCR and immunohistochemistry. The regionally specific action of Nato3 on DA neuron markers suggests that it is regulated by an unknown mechanism that functions early in development within the ventral midbrain. These data provide new insight into the action of Nato3 on DA neuron marker expression in vivo and help to better characterize the role that Nato3 plays in DA neurogenesis.

Disclosures: J.L. Straight: None. **D. Peterson:** None. **M.K. Taylor:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.09/B13

Topic: A.02. Neurogenesis and Gliogenesis

Title: *In vitro* generation of human cortical interneurons for transcriptome analysis

Authors: *J. L. CLOSE[1], S. YAO[2], S. ANDERSON[4], R. DOLMETSCH[3];
[2]Structured Sci., [3]Mol. Networks, [1]Allen Inst. For Brain Sci., Seattle, WA; [4]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Malfunction or loss of GABAergic interneurons has been implicated in multiple human neurodevelopmental and psychiatric disorders, including schizophrenia, autism spectrum disorders and epilepsy. However, little is known about the molecular events that characterize human interneuron differentiation. Here we have established a protocol to efficiently generate human GABAergic cortical interneurons in vitro from human embryonic stem cells and induced pluripotent stem cells. We have characterized the gene expression profile of these cells over time to determine the key molecular events during human interneuron differentiation and find that in vitro-generated interneurons express many of the transcription factors found to be crucial for interneuron differentiation in mouse. Our approach will allow us to identify novel regulators of interneuron differentiation and determine gene expression changes in iPSCs derived from disease patients.

Disclosures: J.L. Close: None. **S. Yao:** None. **S. Anderson:** None. **R. Dolmetsch:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.10/B14

Topic: A.02. Neurogenesis and Gliogenesis

Title: Toll like receptor 5 regulates adult neurogenesis through modulating neural stem cell proliferation and differentiation in hippocampus

Authors: S. CHOI, K. SEONG, M. KIM, J. YANG, M. PARK, J. JUNG, *W.-J. KIM;
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Abstract: Recent reports have showed that several TLR family members were expressed in mouse embryonic/ adult stem cells, suggesting that these may play a role in stem cell activities. To address the possibility that TLR5 plays a functional role in neural stem/progenitor cell proliferation and differentiation from adult mouse, we checked the expressional pattern of TLR5 with SOX2 (stem cell marker) and DCX (new born neuronal marker). TLR5-positive cells were co-localized with SOX2 and DCX in hippocampal dentate gyrus of adult mouse. To verify a role of TLR5 on neural stem/progenitor cell (NSPC) proliferation, we administrated BrdU in TLR5 KO mouse and wild type mouse. TLR5 KO mouse demonstrated a marked increment in BrdU-positive cells in the hippocampal dentate gyrus 2.5 hrs after single injection compared with wild type mouse. To elucidate effect of TLR5 on neural differentiation, we checked DCX/BrdU or NeuN/BrdU double positive cells. TLR5 KO mouse showed a decrease in DCX/BrdU-double labeled cells and NeuN/BrdU-double labeled cells relative to wild type mouse. When we administrated Flagellin known as TLR5 specific agonist in mouse, BrdU-positive cells are transiently reduced and also BrdU/DCX-positive cells were increased in hippocampal dentate gyrus. Taken together, our data suggest that TLR5 plays a crucial role in adult neurogenesis from the hippocampal dentate gyrus in mouse.

Disclosures: S. Choi: None. **K. Seong:** None. **M. Kim:** None. **J. Yang:** None. **J. Jung:** None. **W. Kim:** None. **M. Park:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.11/B15

Topic: A.02. Neurogenesis and Gliogenesis

Title: Differential regulation of histaminergic neurons by dopaminergic signaling pathways in zebrafish larval brains

Authors: *Y.-C. CHEN, P. PANULA;
Univ. of Helsinki, Helsinki, Finland

Abstract: Parkinson's disease with severe brain damage in the nigrostriatal dopaminergic system is also characterized by increased histamine level and histaminergic innervation in the substantia nigra. However, the mechanism of this pathological alteration is unknown. Dopamine interacts with five dopamine receptors. The D1-like receptors comprise D1 and D5 receptors coupled to stimulatory subsets of G proteins to activate adenylyl cyclase. The D2-like receptors including D2, D3, and D4 receptor are coupled to inhibitory G proteins, which inhibit cAMP synthesis. To investigate which subtype of dopamine receptors may modulate the histaminergic neuron development, 1-dpf fish embryos were treated with L-dopa (dopamine precursor), SKF38393 (D1-like receptor agonist), quinpirole (D2-like receptor agonist) and haloperidol (D2-like receptor antagonist) until 5 dpf. The drug effect on the histaminergic neurons was studied by counting histamine-ir and hdc expressing cells. We found that in L-dopa, quinpirole and SKF38393 treated groups the number of histaminergic cells in the caudal hypothalamus was significantly decreased, whereas the number of TH1-ir cells was increased in the diencephalic region including TH1 cell groups 8 to 13 compared with the sham treatment. Haloperidol treatment did not alter the number of the histaminergic neurons. Furthermore, the quinpirole administration restored the decrease of histaminergic neurons in th1-deficient fish brains. On the other hand, L-dopa and quinpirole treatments neutralised the increase of histaminergic neurons in th2-deficient brains. We also found that overexpression of Wnt antagonist dickkop 1 increased dopaminergic neurons in the diencephalon whereas the histaminergic neurons were significantly reduced. These results indicate that the overactive dopamine system has a negative effect on the histaminergic neuron development; in contrast, lack of dopamine raises the histaminergic neuron numbers, suggesting that dopamine is involved in the regulation of the histaminergic system during zebrafish development.

Disclosures: Y. Chen: None. **P. Panula:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.12/B16

Topic: A.02. Neurogenesis and Gliogenesis

Support: MICINN FONDOS FEDER REF. BFU2010-27326

GENERALITAT VALENCIANA – PROMETEO /2009/028

RD06/0010/0023

RD12/0019/0024

CSD2007-00023

Title: Study of the basal forebrain in LIS1 mutant mouse

Authors: *A. POMBERO[1], R. GARCIA-LOPEZ[1], O. REINER[2], R. TABARES-SEISDEDOS[3], S. MARTINEZ[1];
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Abstract: *LIS1* is one of principal genes related with Type I lissencephaly, a severe human brain malformation characterized by an abnormal neuronal migration in the cortex and underlaying predisposition to develop mental disorders. The role of this gene has been studied using the *Lis1/sLis1* murine model, which has deleted the first coding exon from the *Lis1* gene. Homozygous mice are not viable but heterozygous have shown abnormal neuronal morphology and cortical dysplasia, hippocampal abnormalities and enhanced excitability. *Lis1/sLis1* embryos also exhibited a delay of cortical innervation by the thalamocortical fibers. Moreover, it has been suggested that the maturation of cortical neurons plays an important function in the incursion of thalamocortical axons in the developing cortex. Basal forebrain cholinergic neurons migrate from pallium to subpallium and represent the main cholinergic input to the cerebral cortex. Interestingly, this projection plays a crucial role in modulating cortical activity and facilitating processes of attention, learning, and memory; and has not been studied in *Lis1/sLis* mice. We hypothesized that disorganized cortex and hippocampus could affect cholinergic projections from the basal forebrain and septum, respectively, in *Lis1/sLis1* mouse. Here we first studied the basal forebrain in *Lis1/sLis1* mice during development and observed significant structural and hodological differences between wild-type and *Lis1/sLis1* embryos. In addition, septo-hippocampal projections showed a delayed development in mutant embryos. This retard may be related with the cell disorganization of the dentate gyrus in the same way that cortical plate neurons are related with the invasion of thalamocortical axons. Basal forebrain abnormalities

could contribute to explain the enhanced brain excitability and deficits in hippocampal-dependent learning behaviors in *Lis1/sLis1* mutant mice and comorbidity of cognitive symptoms associated to mental diseases.

Disclosures: A. Pombero: None. **R. Garcia-Lopez:** None. **O. Reiner:** None. **R. Tabares-Seisdedos:** None. **S. Martinez:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.13/B17

Topic: A.02. Neurogenesis and Gliogenesis

Support: MEXT, JSPS KAKENHI Grant Numbers 1950026

MEXT, JSPS KAKENHI Grant Numbers 21249013

MEXT, JSPS KAKENHI Grant Numbers 250028

Innovative Cell Biology by Innovating Technology from the Japan Science and Technology Agency

Core Research for Evolutional Science and Technology (CREST) grant from the Japan Science and Technology Agency

Title: RNA Binding Protein Sfpq is required for the expression of neuron-specific long pre-mRNAs essential for brain development

Authors: *A. TAKEUCHI[1], K. IIDA[1], K. NINOMIY[1], M. ITO[2], K. OHNO[2], M. HAGIWARA[1];
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Abstract: Recent methodological advances using microarray, deep-sequencing and biochemical analysis combining bioinformatics provide growing evidence for the essential roles of mRNA processing on the neural development. The mRNA processing is mediated by the RNA-binding proteins which regulate the expression of many genes co-transcriptionally or post-transcriptionally through direct interaction with pre-mRNAs. Here we found that RNA-binding protein, Sfpq (splicing factor, proline/glutaminerich) plays an essential function in mammalian

brain. In embryonic mouse brains, Sfpq is specifically expressed in nascent cortical plate neurons after they differentiate and migrate from neuronal progenitor cells, suggesting its crucial functions for the development of cerebrocortical neurons. To address the in vivo function of Sfpq, we disrupted the Sfpq gene in mice brain. Neuronal tissue specific knockout of Sfpq caused apoptosis in neurons and massive loss of brain tissues in the developing brains including neocortex, indicating that Sfpq is essential for differentiation or maturation of neurons. Next, we produced a specific antibody against Sfpq, and conducted the iCLIP analysis to identify the target of Sfpq in the mouse embryonic neocortex. Distribution of the iCLIP tags showed saw-tooth patterns on entire pre-mRNAs of more than 7400 genes with low sequence specificity. The density of the tags was highest in 5' end of introns, and gradually declined to 3' end. In these large populations of Sfpq-binding genes, only specific gene subsets which express long pre-mRNAs were significantly down-regulated in the Sfpq-disrupted mice brain. According to the Gene Ontology, these genes have essential functions for the brain development, such as cell-adhesion, cell motion, axon guidance, ion channel activity or receptor related molecules, and synaptic vesicle transport and related molecules. Our comprehensive transcriptome analysis showed that 17.4% of specifically expressed pre-mRNAs exceed 262k in differentiated neurons. These data indicate that the RNA binding protein Sfpq is required for the expression of long pre-mRNAs which play essential roles for the survival of neurons, especially in the cortical plate at the developmental stages of brain.

Disclosures: A. Takeuchi: None. **K. Iida:** None. **K. Ninomiy:** None. **M. Ito:** None. **K. Ohno:** None. **M. Hagiwara:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.14/B18

Topic: A.02. Neurogenesis and Gliogenesis

Support: NWO-ALW 816.02.012 (to M.P.S)

NWO-VICI 865.09.002 (to M.P.S.)

Title: En1 and Pitx3 interplay in dopaminergic subset-specification

Authors: *J. VEENVLIET[1], M. T. M. ALVES DOS SANTOS[2], W. M. KOUWENHOVEN[1], L. VON OERTHEL[1], J. L. LIM[2], A. J. A. VAN DER LINDEN[2], M. J. A. GROOT KOERKAMP[3], F. C. P. HOLSTEGE[3], M. P. SMIDT[1];
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Abstract: Mesodiencephalic dopaminergic (mdDA) neurons control locomotion and emotion and are affected in multiple psychiatric and neurodegenerative diseases, including Parkinson's Disease (PD). Homeodomain transcription factor Pitx3 is pivotal in mdDA neuron development and loss of Pitx3 results in programming deficits in a rostrolateral subpopulation of mdDA neurons destined to form the Substantia Nigra (SNc), reminiscent of the specific cell loss observed in PD. We show here that in adult knock-out mice for a second homeoprotein, Engrailed-1 (En1), dramatic loss of mdDA neurons and striatal innervation defects were observed, partially reminiscent of defects observed in Pitx3(-/-) mice. We then continue to reveal developmental crosstalk between En1 and Pitx3 through genome-wide expression analysis. During development both En1 and Pitx3 are required to induce expression of mdDA genes in the rostrolateral subset destined to form the SNc. In contrast, Pitx3 and En1 reciprocally regulate a separate gene cluster, including Cck, demarcating a caudal mdDA subset in wild-type embryos. Whereas En1 is crucial to induce this caudal phenotype, Pitx3 antagonizes it rostrolaterally. The combinatorial action of En1 and Pitx3 is potentially realized through at least three levels of molecular interaction: 1) influencing each others' expression level 2) release of HDAC-mediated repression of Nurr1 target genes and 3) modulating En1-activity through Pitx3 driven activation of En1 modulatory proteins. These findings show how two critical mediators of mdDA neuronal development, En1 and Pitx3, interplay in dopaminergic subset-specification, underlying specific molecular features, exemplified by specific vulnerability of the SNc as found in PD.

Disclosures: J. Veenvliet: None. **M.T.M. Alves dos Santos:** None. **W.M. Kouwenhoven:** None. **L. von Oerthel:** None. **J.L. Lim:** None. **A.J.A. van der Linden:** None. **M.J.A. Groot Koerkamp:** None. **F.C.P. Holstege:** None. **M.P. Smidt:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.15/B19

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH RO1 NS037756

Title: Role of notch pathway in development and survival in chronic hypoxia

Authors: *P. AZAD[1], J. BROPHY[2], G. HADDAD[3];
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Abstract:
Oxygen is essential for the development and growth of all organisms. Our research is focused on understanding the molecular mechanisms that lead to injury or adaptation to hypoxic stress using *Drosophila* as a model system. To identify the genes involved in hypoxia tolerance, we screened P-element insertion lines generated by Gene Disruption Project. For hypoxia tolerance, we screened for a) eclosion rates after following the development of embryos placed in 5% O_2 to eclosion and adulthood, b) number of adult flies surviving after eclosion .Out of 2187 lines screened,44 P-element lines had eclosion rates significantly higher (>70% eclosion) than the controls (eclosion rate ~7-8%) under hypoxia. The molecular function of these lines ranged from cell cycle regulation, DNA or protein binding, ATPase activity, and transcriptionalco-regulators. In this screen, we found certain interesting candidate genes such as *sec8, cyclin E, osa, l(3)mbn, lqf* which show tremendous hypoxia tolerance during all stages of development. By bioinformatic analysis, we found that 20 out of the 44 candidate genes are linked to Notch signaling pathway, strongly suggesting that this pathway is essential for hypoxia tolerance in flies. Furthermore, we employed the UAS-Gal4 system to further dissect the role of these genes ubiquitously or in specific tissues in vivo. We found that the specific knock-down of osa in the nervous system (elav-gal4) and mushroom body (MB) significantly decreased eclosion rates. This suggests that *osa* has a specific rolein the central nervous system and under hypoxia its loss of function decreases eclosion rates. We also observed that up-regulation of *lqf* in glial cells leads to a significantly higher eclosion (93%,$P<0.001$). Our real-time PCR analysis shows that several Notch pathway genes such as *Delta* and *Enhancer of Split α* are significantly altered in the P-element lines of *lqf* and *osa* genes reinforcing our hypothesis that Notch activation plays a critical role in development under hypoxic conditions. Furthermore, by using a series of genetic crosses (using UAS and RNAi lines) we are studying the effects of activation or inhibition of Notch pathway genes on hypoxia tolerance in *lqf* gene double mutants ubiquitously and in specific tissues to prove the functional link. In flies, Notch influences the fate of cells in both the central nervous system (CNS) and peripheral nervous system (PNS). Interestingly, we found that regulation of single genes such *lqf* gene (Notch regulator via Delta ligand) in glial cells and *osa* gene in nervous system can play an important role in development during hypoxia.

Disclosures: P. Azad: None. **J. Brophy:** None. **G. Haddad:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.16/B20

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIDA R21DA031557 to SD

Title: DNA methylation profiling of human cortical GABAergic neurons revealed by a novel flow cytometry-based nuclei separation approach

Authors: *A. KOZLENKOV[1,2], S. RUDCHENKO[3], M. WEGNER[4], M. BARBU[3], M. BIBIKOVA[5], Y. HURD[1], S. DRACHEVA[1,2];
[1]Dept. of Psychiatry, Mount Sinai Sch. of Med., New York, NY; [2]Psychiatry Res., James J. Peters VA Med. Ctr., Bronx, NY; [3]Hosp. for Special Surgery, New York, NY; [4]Lehrstuhl für Biochemie und Pathobiochemie, Inst. für Biochemie, Erlangen, Germany; [5]Illumina, Inc., San Diego, CA

Abstract: To date, the majority of the epigenetic studies of the human brain have used bulk tissue specimens, and, therefore, did not allow discerning specific contributions of various cellular populations. However, recently it has become apparent that there are significant differences in epigenetic profiles among different cell populations present in the adult brain, which warrants their in-depth epigenetic characterization. This task is especially important for the study of cortical GABAergic neurons. Whereas this group of neurons has been consistently implicated in many neurological disease conditions, the relatively small proportion of this population (~20% of all neurons in human cortex) implies that its contribution to the epigenetic profiles obtained from the bulk brain tissue can be easily masked by the contributions of other cell types in the brain.
Based on the existing nuclei sorting protocol that employs anti-NeuN antibodies to separate neuronal and glial nuclei from the postmortem human brain, we developed a novel multicolor FACS-based approach to isolate nuclei of a subpopulation of human cortical GABAergic neurons. The collected fraction was strongly enriched in neurons which developmentally derive from the medial ganglionic eminence (MGE) and include parvalbumin (PVALB)- and somatostatin (SST)-positive GABAergic neurons. In agreement with the previously published data, the isolated subpopulation comprised ~15% of all neurons. To validate the specificity of the method, we isolated RNA from sorted nuclei and confirmed by qPCR that the isolated subpopulation showed a strong enrichment of GABAergic-specific transcripts, such as *GAD1*, *PVALB*, *SST* and *LHX6*, and depletion of markers of glutamatergic neurons. We next obtained the genome-wide DNA methylation profile of the MGE-derived human cortical interneurons and compared it with that of the remaining neuronal fraction, which predominantly consisted of glutamatergic projection neurons. Thousands of CpG sites were found to be differentially

methylated between the two neuronal subpopulations, often displaying gene-specific localization. Differentially methylated sites were depleted from CpG islands and promoters. In contrast, cell type-specific differential methylation was overrepresented within distal regulatory elements. The current study opens previously unavailable opportunities to study epigenetic properties of GABAergic neurons in autopsy brains from individuals with various psychiatric conditions.

Disclosures: A. Kozlenkov: None. **S. Dracheva:** None. **M. Bibikova:** None. **S. Rudchenko:** None. **M. Barbu:** None. **Y. Hurd:** None. **M. Wegner:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.17/B21

Topic: A.02. Neurogenesis and Gliogenesis

Support: Oklahoma State Uiversity CHS

Title: Neuronal insulin regulated nestin expression when compared to IGF-1 in neuron cell cultures obtained from stem cells of fetal rat brain

Authors: *R. SCHECHTER, K. E. MILLER;
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Abstract: Insulin and insulin like-growth factor 1 **(IGF1**) "de novo" production by neurons has been proven by us and other laboratories. Neuronal insulin [**I(n**)] induces axonal growth and differentiation, decreases nestin positive neurons and promotes neurofilament expression. **IGF1** does not promote axonal growth, decreases neuronal differentiation, but induces neuronal survival. We quantitated the role of **I(n)** and **IGF1** on nestin expression in the axons using stem cells from fetal rat brain neuron cell cultures (**NCC**). NCC were isolated from 16 days gestational age fetal rat brain with a mouse monoclonal p-75 antibody, followed by 280 µm magnetic beads conjugated to goat anti-mouse antibody. NCC were cultured in serum free-insulin free DMEM /F12 medium (IFM), insulin media (IM) (5 ng/ml), IGF1 medium (100 ng/ml) or 5 µl/ml of a guinea pig anti-insulin antibody. Nestin was identified at 1 and 3 days of culture employing a mouse monoclonal anti-nestin antibody, followed by a goat anti-mouse antibody conjugated with FITC. Nestin was seen in neuron bodies and along axons at day 1 and day 3 of incubation. Quantitation of nestin within the IFM showed a significant decreased ($p<0.05$) at day 3 of incubation when compared to day 1. Nestin was significantly decreased ($p<0.05$) at day 1 of incubation with IM when compared to day 1 of IFM. Neurons incubated

with anti-insulin antibody for 3 days showed no significant differences in nestin concentration compared to IFM day 1, but a significant increase ($p<0.05$) was found compared to IFM day 3 and IM. Nestin quantitation showed a significant increase ($p<0.05$) within the IGF1 medium at day 1 and 3 of incubation when compared to IFM day 3 and IM. No significant difference ($p>0.05$) was found between IGF1 media, IFM day 1 and anti-insulin antibody treatment. Thus, **I(n)** promotes neuron differentiation by negatively regulating nestin. IGF1 may have a role of promoting neuronal survival, but not differentiation. Lack of insulin may induce alterations in neurons in the balance of the neuronal filaments. This is important in Alzheimer's disease associated with Diabetes Mellitus, Type I Diabetes Mellitus and Alzheimer's disease in which alterations of cytoskeleton are seen. This study and the in vivo results are in favor of insulin being produce by neurons. **I(n)** role within the brain based in the current and previous results is to regulate neuronal cytoskeleton expression. The concentration of insulin needs to be constant within the brain for this function. A "de novo" synthesis of insulin by neurons provides a constant source of insulin. This may be evident by the short half life of insulin and that pancreatic insulin secretion is induced in an episodic fashion by glucose.

Disclosures: R. Schechter: None. **K.E. Miller:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.18/B22

Topic: A.02. Neurogenesis and Gliogenesis

Support: KMSIP Global Research Laboratory Program (2009-00424)

KMSIP Brain Research Program (2009-0081465)

KMSIP Drug Target Validation (2010-0029943)

KMSIP Stem Cell Research (2006-2004289)

Title: Retinal pigment epithelium is a Notch signaling niche in mouse retina

Authors: T.-J. HA[1], S. KIM[1], J. HATAKEYAMA[2], K. SHIMAMURA[2], Y.-Y. KONG[3], *J. KIM[1];
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Abstract: To maintain vertebrate neural progenitor cell (NPC) fate, Notch is activated in NPC by its ligands expressed in adjacent cells, of which identities are largely unknown yet. To elucidate the sources of functionally active Notch ligands in mammalian retina, we negatively modulated Notch ligand activities by specific elimination of Mindbomb1 (Mib1), an ubiquitin ligase activating Notch ligands, in retinal progenitor cells (RPCs), post-mitotic retinal neurons (PMNs), or retinal pigment epithelium (RPE). The Mib1-deficient RPCs failed to induce Notch activation in neighboring RPCs, which prematurely differentiate into retinal neurons. The Mib1 in RPE was also crucial for Notch activation in RPC, whereas the Mib1 in PMNs was dispensable. We found RPE expresses Notch ligands at apical membrane that adheres to Notch1-enriched RPC apical membrane. Elimination of Notch1 and Notch2 in RPE resulted in insignificant changes, while hyperactivation of Notch signaling in RPE facilitated ciliary epithelial fate, suggesting that Notch signaling in RPE should be suppressed for supporting retinal development in non-cell autonomous manners. Together, we propose a model that a RPC acquires its fate by receiving extra Notch signals from RPE, in addition to a reciprocal Notch signaling between post-mitotic RPC daughter cells.

Disclosures: T. Ha: None. **S. Kim:** None. **J. Hatakeyama:** None. **K. Shimamura:** None. **Y. Kong:** None. **J. Kim:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.19/B23

Topic: A.02. Neurogenesis and Gliogenesis

Support: Sandler Foundation

Title: Gene coexpression analysis identifies a molecular signature of migrating GABAergic neurons in developing human neocortex

Authors: *C. S. RAJU[1], A. STANCO[2], J. L. R. RUBENSTEIN[2], M. C. OLDHAM[1];
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Abstract: The neocortex is an extraordinarily complex, heterogeneous structure, comprised of many different cell types. During neocortical development, radial glia generate excitatory neurons that migrate radially to form the six cortical layers, followed by glial cells. In contrast, GABAergic neurons are largely derived from the ganglionic eminence in the ventral

telencephalon, and arrive in the neocortex via long-distance tangential migration. GABAergic dysfunction has been implicated in a variety of neurological disorders; it is therefore critical to understand the molecular basis for the proper specification and migration of GABAergic neurons. We have developed an unbiased method called Gene Coexpression Analysis of Serial Sections (GCASS) that can identify molecular signatures of distinct cell types in a single tissue sample. Using this method, we analyzed gene coexpression relationships in the developing neocortex of an 18 gestational week human individual and identified a molecular signature of migrating GABAergic neurons. We validated the specificity and reproducibility of this signature through immunostaining and gene coexpression analysis of several independent transcriptomic datasets generated from fetal human neocortical samples, demonstrating that it is robust across a variety of sampling strategies and technology platforms. Furthermore, we compared this signature with microarray data generated from GABAergic neurons obtained via FACS of neocortical samples from P0 CXCR7-GFP+ mice, identifying conserved and distinct patterns of gene expression between the species. Our results implicate a large number of novel genes as candidates involved in the differentiation and migration of GABAergic neurons, and offer a strategy for exploring aspects of interneuron development that may distinguish humans from other species.

Disclosures: C.S. Raju: None. **A. Stanco:** None. **J.L.R. Rubenstein:** None. **M.C. Oldham:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.20/B24

Topic: A.02. Neurogenesis and Gliogenesis

Support: NCNPR-USDA-ARS Cooperative Scientific Agreement # 58-6408-2-0009

NCRR Grant Number 5P20RR021929 (CORE-NPN)

Title: *In vitro* neurotrophic properties of suberoylanilide hydroxamic acid (SAHA), a pan histone deacetylase inhibitor

Authors: *S. SHUKLA[1], B. L. TEKWANI[2], L. A. WALKER[2];
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Abstract: Degeneration of neurons is the major cause of the neurodegenerative diseases. Neurodegenerative diseases including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD) and Amyotrophic Lateral Sclerosis (ALS) occur as a result of progressive loss of structure or function of neurons, including death of neurons. Neurotrophic actions and neuroprotection leading to neuroplasticity and neurite outgrowth are important markers for neuroregeneration. Neuritogenesis or neuritic outgrowth is a fundamental process in the differentiation of neurons and plays an important role in neuronal development and formation of synapses. These processes are regulated by extrinsic and intrinsic determinants that affect gene expression profiles and signal transduction pathways. Within the past decade, there have been many studies showing neuroprotective effects of inhibitors of histone deacetylases (HDAC). Evaluation of a battery of HDAC inhibitors showed promising neurotrophic effects of SAHA, a pan HDAC inhibitor that binds to the catalytic site of the enzyme, inhibits class I and class II HDACs, arrests cell growth in a wide variety of transformed cells in culture and the first HDAC inhibitor approved by FDA for treatment of chronic T-cell leukemia. The neurotrophic properties of SAHA were investigated in detail employing NeuroScreen1 (NS-1) cell line, derived from PC12 (rat adrenal pheochromocytoma) cells. NS-1 cells were treated with SAHA for 72 hrs. Cytotoxicity was measured and neurite outgrowth was monitored as neurite numbers and lengths by digital microscope imaging and NIS element software. SAHA independently produced significant increase in neuritic outgrowth. Further, to investigate the mechanism for SAHA-induced differentiation of NS-1 cells the MEK1/2 inhibitors (PD98059 and U0126) and PI3K inhibitor (LY294002) were tested. The neurotrophic actions of both NGF and SAHA were almost completely abolished by co-treatment of the NS-1 cells with the inhibitors of MEK1/2 & PI3K. SAHA produced selective time-dependent hyperacetylation of histones as well as non-histone proteins in NS-1 cells as probed by anti-acetylated lysine antibody. Treatment of NS1 cells with SAHA induced phosphorylation of ERK1/2 at 4 hrs confirming role of ERK pathway in differentiation. In conclusion, SAHA exerts neurotropic action via activation of MEK1/2 & PI3K pathways. Bioactive small molecules with neurotrophic and neuritogenic actions, like SAHA identified in the present study, hold great promise as therapeutic agents for treatment of neurodegenerative diseases and neuronal injuries by virtue of their ability to stimulate neuritic outgrowth.

Disclosures: S. Shukla: None. **B.L. Tekwani:** None. **L.A. Walker:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.21/B25

Topic: A.02. Neurogenesis and Gliogenesis

Support: NEI-5R00EY019547

Title: An alternative splicing factor Celf4 is required for early retinal neuronal differentiation

Authors: A. R. BANDAY, *R. KANADIA, S. CONGDON;
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Abstract: RNA-binding proteins are reported to be involved in several complex neurological diseases. Recently a RNA-binding protein Celf4, which is both an alternative splicing factor and a RNA-transporter, has been implicated in epilepsy disorder in human and mice. In the mouse brain Celf4 is expressed in excitatory neurons of the cerebral cortex and hippocampus. However, the role of celf4 in the development and function of the retina remains unexplored. Here, we have examined the expression pattern and possible role of Celf4 in the developing mouse retina. We analyzed the expression of Celf4 by RT-PCR across the mouse retinal development and found two alternatively spliced isoforms that are unique to the retina. To visualize the expression of Celf4 mRNA and protein in the different cell types of the developing retina we employed in situ hybridization and immunoflourescence (IF), respectfully. We found at embryonic day 12 (E12) and E14 that Celf4 was expressed in the nucleus of retinal ganglion cells. Later in the retinal development from E18 through postnatal day (P14) Celf4 expression was observed both in the nucleus and cytoplasm in ganglion (E16), amacrine (E16), bipolar (P8) and photoreceptor cells (P8). However, in the adult mouse, Celf4 expression is entirely cytoplasmic, suggesting that its role in alternative splicing might be restricted to pre-natal development. These distinct roles of Celf4 may be assigned to the two isoforms that were found in the retina. Specifically, it might be regulated via differential localization and /or post-translation modification site such as phosphorylation. Interestingly, bioinformatics analysis revealed that there was a potential phosphorylation site for PKC-alpha at Serine 164 on the larger retinal isoform (Isoform A) but not on the smaller isoform (Isoform B). Preferential phosphorylation could confer different functions to the two isoforms.
In order to investigate the function of Celf4 in the mouse developing retina, we used constitutively knocked out (KO) mice. Our preliminary analysis showed that in the KO retina at E16, there was a failure to close the retinal fissure, which results in retinal coloboma observed at birth. In all, this suggests that celf4 plays an important role in normal retinal development. In the future, we will employ a conditional KO mouse, to further understand the function of celf4 by identifying its targets in embryonic and postnatal retinal development.

Disclosures: A.R. Banday: None. **R. Kanadia:** None. **S. Congdon:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.22/B26

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant NS066053

Title: The RNA-binding protein motif protein 3 (RBM3) regulates the LIN28-let-7 axis and neural differentiation

Authors: A. GROMOVA[1], C. SPEVAK[2], J. PILOTTE[1], *P. VANDERKLISH[1];
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Abstract: Neurogenesis is a fundamental process underlying brain development and plasticity, and it is dysregulated in a broad spectrum of psychiatric and degenerative conditions. Multiple steps in the process of neurogenesis are critically influenced by microRNAs (miRNAs) - non-coding RNAs that regulate mRNA translation - and by RNA-binding proteins (RNA-BPs) that regulate miRNA biogenesis. A pivotal role in early neurogenesis is played by LIN28, an RNA-BP that suppresses the biogenesis of let-7 miRNAs that target de-differentiation factors, thus maintaining pluripotency. The mRNA encoding LIN28 is itself targeted by let-7, thus creating a "bi-stable switch" wherein either LIN28 or let-7 dominates to favor pluripotency or differentiation, respectively. Signaling pathways that regulate this switch, and the biogenesis of other miRNAs controlling neurogenesis, are thus of fundamental importance in neural development and plasticity. In prior work, we found that the RNA-binding motif protein 3 (RBM3) - an RNA-BP induced by cell stress - is highly expressed in stem/progenitor cells and strongly promotes the biogenesis of all let-7 family members, as well as other miRNAs target LIN28. Based on these findings, we hypothesized that RBM3 modulates the state of the LIN28-let-7 axis to promote neural differentiation. Using the P19 embryonic carcinoma cell line and both mouse and human embryonic stem cells, we found that induction of RBM3 expression promoted let-7 biogenesis and, accordingly, reduced LIN28 expression. Conversely, siRNA-mediated knockdown of RBM3 resulted in reduced let-7 biogenesis and increased LIN28 expression. In the retinoic acid (RA) model of P19 neural differentiation, RBM3 was specifically upregulated during the neurosphere stage, correlating with increased let-7 biogenesis. Knockdown of RBM3 attenuated RA-induced neural differentiation, whereas overexpression of RBM3 promoted it, even in the absence of a neurosphere step and RA. Subsequent studies revealed that the effects of RBM3 on the LIN28-let-7 axis are negatively regulated by post-translational modifications of RBM3 mediated by kinase and protease pathways that contribute to pluripotency. Preliminary analyses of a *Rbm3* KO mouse revealed lower cell counts in hippocampus, and evidence of reduced neurogenesis. Overall, our data suggest that RBM3 is an

important regulator of neurogenesis through its effects on miRNAs, with the apparent capacity to override the influence of LIN28 during early stages of cell commitment. The fact that RBM3 is induced by a wide range of cellular stresses and disease states suggests that it may mediate some of the known effects of these states on neurogenesis.

Disclosures: A. Gromova: None. **C. Spevak:** None. **P. Vanderklish:** None. **J. Pilotte:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.23/B27

Topic: A.02. Neurogenesis and Gliogenesis

Title: VEGF-C/VEGFR3 signaling in adult hippocampal neural stem/progenitor cells and response to physical exercise

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Abstract: Vascular endothelial growth factor family molecules (VEGFs) and their receptors (VEGFRs) are essential for blood vessel and lymphatic vessel growth. Previous studies from our laboratory revealed that VEGF-C, a member of VEGF family, can affect the developing and adult central nervous system by promoting the activity of neural stem/progenitor cells. In the adult subventricular zone, this effect is directly mediated on neural stem/progenitor cells by VEGFR-3, the specific VEGF-C receptor (Genes Dev, 2011). It remained to be determined whether this function extended to other neural stem cell (NSC) populations of the adult brain, and what its physiologic consequences were. We have therefore investigated VEGF-C/VEGFR-3 signaling in the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampal formation, which is one of the specialized regions in which new neurons are generated from NSCs in the adult. The hippocampus is moreover a key regulatory center of cognitive functions and mood. Using BAC-Vegfr3::YFP reporter mice, we detected a specific Vegfr3 expression in SGZ NSCs and early progenitor cells, but not in differentiated neuronal cells. Overexpression of VEGF-C by intra-hippocampal delivery of adeno-associated virus (AAV9)-VEGF-C increased cell division of SGZ VEGFR-3-stem/progenitors without a pro-angiogenic effect. Following NSC-specific deletion of Vegfr3 by tamoxifen treatment of Glast-CreERT2, Vegfr3 flox/flox mice SGZ neurogenesis was not affected in basal conditions. However, neurogenesis was impaired in

response to voluntary exercise activity, which is known to activate quiescent NSCs. Exercise activity was associated with increased levels of VEGFR-3 ligand (VEGF-C/D) expression in the DG. We are currently exploring whether VEGF-C/VEGFR-3 might regulate the positive effect of physical exercise on mood, which has already been suggested to be SGZ-neurogenesis-dependent. Preliminary studies in the rat indicate that mood state is impaired following local delivery of AAV9-VEGF-R3-Ig, which decreases the availability of hippocampal VEGF-C and D.
These results confirm VEGF-C/VEGFR-3 signaling as a hallmark of neural stem/progenitor cells in the adult brain. Our data also suggest that this signaling pathway contributes to mediate the specific response and activity of SGZ neural stem/progenitors to physical exercise, with possible consequences on mood.

Disclosures: J. Han: None. **K. Baker:** None. **N. Fournier:** None. **C. Calvo:** None. **R. Duman:** None. **A. Eichmann:** None. **J. Thomas:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.24/B28

Topic: A.02. Neurogenesis and Gliogenesis

Title: Functional roles and regulation of PTPs during neuronal differentiation

Authors: *B.-S. HAN, S.-Y. KIM, W.-K. KIM, K.-H. BAE, S.-C. LEE;
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Abstract: The phosphorylation status of tyrosine residues on proteins is fundamentally important for the control of different functions of the cells and is the results of the balance between protein tyrosine kinases (PTK) and protein tyrosine phosphatases (PTP). Improper control of tyrosine phosphorylation levels has been linked to a variety of diseases. Several reports have provided evidences that PTPs play an important role in neuronal morphogenesis, neuronal development, axonal guidance, and synaptogenesis. Although important advances have been made in this field, it still needs to be addressed in more detail about the signaling mechanisms involved in this processes. In this study, we performed to investigate PTPs gene expression profiling from mouse embryonic stem cells (J1 ES) to J1 ES derived neurons. Neurons generated from undifferentiated J1 ES cells showed neuronal morphology such as neurite outgrowth, and expressed neuronal markers such as microtubule-associated protein 2, β-

tubulin type III. During neuronal differentiation, several PTPs showed differentially expressed patterns between ES cells and ES derived neurons. Functional studies of PTPs provide a valuable clue for the understanding of neurogenesis mechanism.

Disclosures: B. Han: None. **S. Kim:** None. **K. Bae:** None. **S. Lee:** None. **W. Kim:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.25/B29

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant MH094670 (to MMB and JRE)

NIH Grant NINDS K99NS080911 (to EAM)

Howard Hughes Medical Institute (to TJS and JRE)

Gordon and Betty Moore Foundation (to JRE)

NIH Grant HG006827 (to CH)

Australian Research Council Future Fellowship (to RL)

NIH Grants AI44432 and HD065812 (to A.R.)

Title: Global reconfiguration of neuronal and glial DNA methylation during mammalian brain development

Authors: *E. A. MUKAMEL[1,2], R. LISTER[5,6], J. R. NERY[6], M. URICH[6], C. A. PUDDIFOOT[7], N. D. JOHNSON[7], J. LUCERO[7], Y. HUANG[8], A. J. DWORK[9,10], M. D. SCHULTZ[6,3], M. YU[11], J. TONI-FILIPPINI[5], W. A. PASTOR[8,12], H. HEYN[13], S. HU[14], J. C. WU[14], A. RAO[8], M. ESTELLER[13], C. HE[11], F. G. HAGHIGHI[9], T. J. SEJNOWSKI[7,4,15], J. R. ECKER[6,15], M. M. BEHRENS[7];
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Abstract: The behavioral and cognitive functions of frontal cortex require the interaction of diverse neurons and glial cells with specific roles arising from their location, connections with other brain cells, as well as each cell's intrinsic, epigenetically defined molecular identity. Dynamic epigenetic changes, including variant histones, histone modifications and DNA methylation, are implicated in brain development, maturation and learning. Among these, DNA methylation is a stable covalent modification that can persist in post-mitotic cells throughout the lifetime and thus confers a capacity for long-term memory of cellular identity. At the same time, the DNA methylation status at each of ~1 billion sites across the genome is potentially an information-rich and flexible epigenetic modification that can be altered by cellular activity and which is implicated in learning and memory. Yet elucidating the role of DNA methylation in brain function has been hampered by the lack of precise knowledge of the genomic distribution of this mark in brain cell types. We will present the genome-wide composition, patterning, cell-specificity and dynamics of DNA methylation at single-base resolution in frontal cortex of humans and mice throughout their lifespan. Extensive methylome reconfiguration occurs during development from fetal to young adult. In this period, coincident with synaptogenesis, highly-conserved non-CG methylation accumulates in neurons, but not glia, to become the dominant form of methylation in the genome. We uncovered surprisingly complex features of brain cell DNA methylation at multiple scales, first by identifying intragenic methylation patterns in neurons and glia that distinguish genes with cell-type specific activity. Second, we find >100,000 developmentally dynamic and cell-type specific differentially CG-methylated regions that are enriched at putative regulatory regions of the genome. Third, we report a novel mCH signature that identifies genes escaping X-chromosome inactivation in neurons. Finally, whole-genome detection of 5-hydroxymethylcytosine (hmC) at single-base resolution revealed that this mark is present in fetal brain cells at locations that lose CG methylation and become activated during development. CG-demethylation at these hmC-poised loci depends on Tet2 and Tet3 activity. Overall, brain cell DNA methylation has unique features that are precisely conserved, yet dynamic and cell-type specific.

Disclosures: E.A. Mukamel: None. **R. Lister:** None. **J.R. Nery:** None. **M. Urich:** None. **C.A. Puddifoot:** None. **N.D. Johnson:** None. **J. Lucero:** None. **Y. Huang:** None. **A.J. Dwork:** None. **M.D. Schultz:** None. **M. Yu:** None. **J. Toni-Filippini:** None. **W.A. Pastor:** None. **H. Heyn:** None. **S. Hu:** None. **J.C. Wu:** None. **A. Rao:** None. **M. Esteller:** None. **C. He:** None. **F.G. Haghighi:** None. **T.J. Sejnowski:** None. **M.M. Behrens:** None. **J.R. Ecker:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.26/B30

Topic: A.02. Neurogenesis and Gliogenesis

Support: Mahidol University

Title: The role of CIP4 in neuroblastoma cell proliferation and differentiation

Authors: P. SUTHIVANICH[1], S. KONGOUN[1], J. KWANTONGDEE[1], K. TAYLOR[2], E. W. DENT[3], *W. SAENGSAWANG[4,1];
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Abstract: CIP4, a member of the F-BAR family of proteins, plays important roles in various cellular events including endocytosis and membrane protrusion in several cell types. In primary cortical neurons CIP4 has been shown to induce actin-dependent veil protrusion between filopodia, which results in inhibition of neurite formation. However, the roles of CIP4 in cell proliferation and neuronal differentiation are not known. In this study we found that CIP4 is highly expressed during a very early stage of mouse brain development (E10.5); a time at which neuronal precursors are actively proliferating. To begin to determine whether CIP4 plays a role during neuronal proliferation and differentiation we used two types of cells whose differentiation can be tightly regulated, mouse cathecholaminergic neuroblastoma (CAD) and dopaminergic neuroblastoma (SH-SY-5Y) cells. We show that CIP4 is highly expressed in both cell types when the cells are actively proliferating but decreases significantly during differentiation. In addition, overexpression of CIP4 resulted in a decrease in neuroblastoma cell differentiation. These results suggest that CIP4 may be playing an important role during an early stage of brain development, possibly by inhibiting neuronal differentiation. Further experiments will determine the underlying mechanisms by which CIP4 appears to inhibit neuronal differentiation.

Disclosures: P. Suthivanich: None. **W. Saengsawang:** None. **S. Kongoun:** None. **J. Kwantongdee:** None. **K. Taylor:** None. **E.W. Dent:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.27/B31

Topic: A.02. Neurogenesis and Gliogenesis

Title: Dopaminergic transcriptional determinants repress non-dopaminergic transcription

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Abstract: A cell's path through development from a pluripotent embryonic stem cell to a specific, differentiated adult cell at a precise location in the body, and with a precise function, involves the complicated task of modelling the genome such that desired transcriptional programs emerge, while undesired programs do not. This task is orchestrated, at least in part, through the temporal action of countless patterning and signalling molecules, temporal cellular clocks, as well as stochastic events, and which is ultimately realised at the genomic level by the coordinated action of transcription factors that direct the transcription of these desired programs, while undesired programs are not transcribed. This poses a challenge particularly in the central nervous system, where hundreds of distinct neuronal populations, each serving a particular role, need to be generated. Moreover, the molecular differences between some of these populations, while imperative to their functions, may be quite subtle. We have investigated the influence of dopaminergic transcription factors on the transcriptome of non-dopaminergic neural subpopulations, where they appear to serve a function to repress transcription of non-dopaminergic genes. This role is most strongly seen for Pitx3, a post-mitotic transcriptional determinant found exclusively in ventral midbrain dopaminergic neurons. The observation that a post-mitotic, dopaminergic specific transcription factor is involved in repression of genes not normally expressed in dopamine neurons invites the theory that even after exiting the cell cycle, the dopaminergic identity is not yet concretely specified, and that at this moment transcriptional determinants continue to both promote the dopaminergic program, and repress undesired programs.

Disclosures: N. Kee: None. **J. Mong:** None. **T. Perlmann:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.28/B32

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH grant R01MH080434 to XZ

NIH grant R01MH078972 to XZ

NIH grant R01NS30255 to NPB

a postdoctoral fellowship from University of Wisconsin Center for Stem Cells and Regenerative Medicine to W.G

Title: Rna binding protein elavl4 regulates proliferation and differentiation of adult neural stem cells

Authors: *W. GUO[1], E. POLICH[1], A. GARDINER[2], N. PERRONE-BIZZOZERO[2], X. ZHAO[1];
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Abstract: Adult neurogenesis in the adult brain has been shown to exist primarily in the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. These two distinct pools of neural stem/progenitor cells (NSCs) generate different subtype neurons and respond independently to environmental cues. However, the mechanisms that differentially control neurogenesis in these two brain regions are still unclear. The RNA binding protein Elavl4 (HuD) is a regulator of neuronal differentiation and involved in paraneoplastic encephalomyelitis disorders with learning deficits. Here, we found HuD has distinct expression patterns in these two neurogenic regions. HuD is expressed throughout the process of SVZ neurogenesis from NSCs to maturation neuron, whereas HuD only expressed in NSCs and doublecortin-positive immature neuron in the DG. We found that HuD exhibit differential regulatory roles in in NSCs derived from these two neurogenic regions. Furthermore, we found HuD may regulate SVZ-NSCs and DG-NSCs differentiation through different molecular mechanisms. Taking together, investigation of differential regulation of SVZ and DG NSCs by HuD not only shed light on a mechanism that governs the different neurogenesis in these two neurogenic zones, but also provide mechanistic insights into paraneoplastic encephalomyelitis.

Disclosures: W. Guo: None. **E. Polich:** None. **A. Gardiner:** None. **N. Perrone-Bizzozero:** None. **X. Zhao:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.29/B33

Topic: A.02. Neurogenesis and Gliogenesis

Support: Istituto Pasteur-Fondazione Cenci Bolognetti

MIUR grants C26A11CC9E and C26N12BZ5K

Title: Post-natal developmental alterations in the retina of dystrophic mdx mice

Authors: *M. DE STEFANO[1,2], I. PERSICONI[1], G. LUPO[1], V. LICURSI[1], N. A. GUADAGNO[1], R. NEGRI[1];
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Abstract: Duchenne muscular dystrophy (DMD) is a severe X-linked myodegenerative disease caused by defective expression of full length dystrophin (Dp427) consequent to mutations in the DMD gene. Besides muscle degeneration, DMD patients also experience forms of cognitive impairment, neurological and autonomic disorders, altered scotopic electroretinogram and red-green color vision defect. In normal retina, different dystrophin isoforms, including Dp427, are present at the ribbon synapses between photoreceptors and bipolar cells, in the inner and outer nuclear layers and in amacrine cells. However, despite several studies, little is still known on how lack of Dp427 and its isoforms induce the described retinal physiological alterations. In this study, we evaluate differences in both morphological differentiation of the retinal layers and gene expression during post-natal development of wild type (WT) and dystrophic *mdx* mouse retina. Discrimination of the different retinal layers, a phenomenon occurring after birth, was analyzed on paraffin-embedded eye sections, from P5, P10 and 6-7 week old mice. The thickness of different layers, i.e. outer segment of photoreceptors (OSP), outer nuclear and plexiform layers, inner nuclear and plexiform layers and ganglion cell layer (GCL), was measured from 3-4 animals/genoptype and statistically compared. A significant reduction in the thickness of both the OSP (where visual pigments are located) and GCL was observed at P5, when retinal cells are still migrating in their final position. Moreover, at this same date, segregation of the different cells in the appropriate layer of the WT mouse retina starts to become evident, while is still confused in the *mdx* genotype, suggesting an early asynchronous migration of retinal cells to their final position. All these gross dissimilarities disappear in older mice. Differences in mRNA expression between the two genotypes were, then, evaluated by RNA Sequencing on P5 mice. Combining edgeR and DESeq methods of analysis, about 50 genes were significantly up or down regulated (by at least one fold) in *mdx* mice compared to WT. The majority of the down regulated genes was related to cell development, transcription, cytoskeleton dynamics and cell stress response. In conclusion, our data suggest that, as in other nervous system regions, also in the retina dystrophin isoforms play a major role in neural development and early differentiation.

Disclosures: M. De Stefano: None. **I. Persiconi:** None. **G. Lupo:** None. **V. Licursi:** None. **N.A. Guadagno:** None. **R. Negri:** None.

Poster

511. **Neuronal Differentiation: Mechanisms II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 511.30/B34

Topic: A.02. Neurogenesis and Gliogenesis

Support: Department of Defense

Hope for Vision Foundation

Pew Foundation

NEI (P30 EY014081)

NIH T32-NS07492

Title: A soxc transcriptional network is required for visual pathway development

Authors: *J. HERTZ[1,3], X.-L. JIN[2], B. A. DEROSA[4], J. Y. LI[1], P. VENUGOPALAN[1], D. A. VALENZUELA[1], R. D. PATEL[1], K. R. RUSSANO[1], S. A. ALSHAMEKH[5], D. VELMESHEV[1], Y. CHENG[1], T. M. BOYCE[1], A. DREYFUSS[1], M. S. UDDIN[1], K. J. MULLER[1], D. M. DYKXHOORN[1], J. L. GOLDBERG[1,3];
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Abstract: The cell-autonomous regulators or extrinsic signals sufficient to specify retinal ganglion cell (RGC) fate in the developing retina or from adult stem cells remain largely obscure. Here we report a new molecular pathway involving Sox4/Sox11 in parallel to GDF-11/Math5 signaling that is required for RGC differentiation from retinal progenitor cells (RPCs) and optic nerve formation in vivo, and sufficient to potentiate the differentiation of both mouse and electrophysiologically active human RGCs from induced pluripotent stem (iPS) cells. We further describe a regulatory network whereby the previously described inhibitor of RGC differentiation, REST, depends on suppression of Sox4 expression, and provide evidence for a novel soluble regulator of RGC differentiation, the TGFß family member GDF-15, which requires Sox4 to induce RGC differentiation. Although our data suggests that Sox4 and Sox11 are independently required for RGC development, the two family members interact such that the normal SUMOylation of Sox11, which decreases its nuclear localization and suppresses its pro-RGC activity, is decreased in the absence of Sox4, allowing Sox11 to compensate for Sox4

absence. These data define a novel molecular network necessary and sufficient for RGC fate specification and suggest a number of novel pro-RGC molecular manipulations which may provide potential promise for cell replacement-based therapies for glaucoma and other optic neuropathies.

Disclosures: J. Hertz: None. **X. Jin:** None. **B.A. DeRosa:** None. **J.Y. Li:** None. **P. Venugopalan:** None. **D.A. Valenzuela:** None. **R.D. Patel:** None. **K.R. Russano:** None. **S.A. Alshamekh:** None. **D. Velmeshev:** None. **Y. Cheng:** None. **T.M. Boyce:** None. **A. Dreyfuss:** None. **M.S. Uddin:** None. **K.J. Muller:** None. **D.M. Dykxhoorn:** None. **J.L. Goldberg:** None.**Poster**

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.01/C1

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: MDA Research Development Grant 186316 to C.W.L.

Hong Kong RGC GRF Grant 662312 to C.W.L. and H.B.P.

Title: Crosslinking-induced endocytosis of acetylcholine receptors by quantum dots

Authors: C. LEE[1,2], H. L. ZHANG[1], L. GENG[1], *H.-M. B. PENG[1];
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Abstract: In majority of patients with myasthenia gravis (MG), anti-acetylcholine receptor (AChR) antibodies are generated in their immune system, which target the postsynaptic AChR clusters and thus compromises membrane integrity of neuromuscular junctions (NMJs) leading to muscle weakness. The antibody-induced endocytosis of AChRs in the postsynaptic membrane represents the initial step in MG pathogenesis; however the molecular mechanisms underlying AChR endocytosis remain largely unknown. Here, we used multivalent quantum dots (QDs) to mimic the pathogenic antibodies for inducing the crosslinking and internalization of AChR from the postsynaptic membrane. By labeling the muscle cells with biotin-conjugated α-bungarotoxin followed by QD-conjugated streptavidin, we were able to differentiate the surface versus internalized AChRs by comparing their size, fluorescence intensity, trajectory, and subcellular localization of the QD signals. These crosslinking-induced internalized AChRs were highly co-localized with an early endosomal marker. QD-induced AChR endocytosis was mediated via clathrin-dependent, and caveolin-independent, mechanisms. Nocodazole or cold temperature treatment, that largely disrupted microtubule structures, arrested the movement of QD-induced

AChR vesicles inside the cells. Furthermore, activation of agrin/MuSK signaling pathway, by either agrin treatment or overexpression of MuSK and rapsyn, significantly suppressed QD-induced internalization of AChRs, suggesting synaptogenic signals increase the stability of surface AChRs. Lastly, QD-induced AChR crosslinking potentiated the dispersal of prepatterned AChR clusters upon synaptic induction. Taken together, this study reports a novel approach to study the trafficking mechanisms of AChRs upon receptor crosslinking and endocytosis, and demonstrates the protective action of agrin-MuSK signaling in the crosslinking-induced AChR endocytosis.

Disclosures: C. Lee: None. **H.L. Zhang:** None. **L. Geng:** None. **H.B. Peng:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.02/C2

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant 1ZIAAA000437

Title: Reversed timing of receptor expression and axon extension during synaptogenesis offers new insight into mechanisms of synaptic transmission

Authors: *M. C. MOTT[1], K. EPLEY[3], J.-Y. PARK[1], G. B. DOWNES[4], C. HARRIS[2], K. BRIGGMAN[2], F. ONO[1];
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Abstract: Synaptogenesis is regulated by a well-defined sequence of events that occur during development. Although pre-synaptic axons were initially considered the leading players in synapse formation, recent studies in the neuromuscular junction (NMJ) demonstrate that post-synaptic development precedes and influences axonal guidance. The functional significance of this temporal sequence remains unknown. In the present study, we leveraged the genetic malleability of zebrafish to artificially reverse the temporal expression of pre- and postsynaptic components. By exploiting the rapid external development and transparency inherent to zebrafish, we could explore the in vivo manifestations of this perturbation.
We generated a transgenic zebrafish line in which the chemical RU486 controlled temporal expression of acetylcholine receptors (AChRs). This inducible system was crossed into a paralyzed mutant zebrafish line lacking postsynaptic AChRs in the NMJ. When AChRs were expressed before the arrival of neuronal axons, synapse formation proceeded normally leading to

the functional rescue of paralyzed larvae and their survival to adulthood. Conversely, larvae in which the timing of axon arrival and receptor expression was reversed displayed compromised locomotion and synapse transmission. Under this condition, AChR localization occurred independent of the presence of neuronal axon terminals. Although nerve terminals were differentiated prior to AChR expression, motor neurons followed postsynaptic cues to guide axons toward newly expressed AChRs. Unexpectedly, electrophysiological recordings revealed a complete lack of miniature end plate currents, despite the presence of evoked postsynaptic currents.
These data provide the first evidence that sequential expression of pre- and postsynaptic components plays a critical role in the function of the developing synapse. In particular, reversing the temporal expression of pre- and postsynaptic components leads to inhibition of spontaneous vesicle release, which to our knowledge has not been observed in any other synapse.

Disclosures: M.C. Mott: None. **K. Epley:** None. **J. Park:** None. **G.B. Downes:** None. **C. Harris:** None. **K. Briggman:** None. **F. Ono:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.03/C3

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH R01 NS072129

NSF IOS-1121095

Title: RPM-1 functions through ANC-1 and beta-catenin to regulate synapse formation and axon termination

Authors: E. D. TULGREN[1], S. M. TURGEON[2], K. J. OPPERMAN[2], *B. GRILL[2];
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Abstract: Mutations in Nesprin-1 and 2 (also called Syne-1 and 2) are associated with numerous diseases including: schizophrenia, autism, cerebellar ataxia, cancer and Emery-Dreifuss muscular dystrophy. Nesprin1 and 2 have conserved orthologs in flies and worms called MSP-300 and abnormal nuclear Anchorage (ANC)-1, respectively. The Nesprin protein family mediates nuclear and organelle anchorage and positioning, as well as Golgi trafficking. In the nervous system, the only known function of Nesprin-1 and 2 is in regulation of neurogenesis and neural

migration. It remains unclear if Nesprin-1 and 2 regulate other functions in neurons. Using a proteomic approach in C. elegans, we found that ANC-1 binds to the Regulator of Presynaptic Morphology (RPM)-1. RPM-1 is part of a conserved family of signaling molecules called Pam/Highwire/RPM-1 (PHR) proteins that regulate axon guidance, axon termination and synapse formation in developing neurons. Studies on invertebrates have shown that the PHR proteins regulate axon regeneration and Wallerian degeneration in mature neurons. We have found that anc-1 regulates axon termination in the mechanosensory neurons, and synapse formation in the GABAergic motor neurons of C. elegans. Our genetic analysis indicates that anc-1 functions through the beta-catenin bar-1. We have also found that the anc-1/bar-1 pathway functions cell autonomously, downstream of rpm-1 to regulate neuronal development. Dominant negative analysis indicated that ANC-1 binding to the nucleus is required for its function in axon termination and synapse formation. Our study highlights a new role for ANC-1 in neuronal development, and unveils a new mechanism by which RPM-1 functions. At present, our findings represent the first genetic link between RPM-1 and a pathway that is regulated by extracellular signals, such as Wnts.

Disclosures: E.D. Tulgren: None. **S.M. Turgeon:** None. **K.J. Opperman:** None. **B. Grill:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.04/C4

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant NS047484

NIH Grant MH099082

Title: Wnt signaling in the formation of neuromuscular junction

Authors: *L. WANG, Y. ZOU;
UCSD, San Diego, CA

Abstract: Wnt signaling plays important roles in axon guidance and synapse development. However, its functions in the guidance of phrenic nerve and the formation of neuromuscular junction (NMJ) are not well understood. Here we explore the function of Wnt signaling using several genetic tools. We first analyze the overall termination patterns of the phrenic nerve and found that acetylcholine receptor (AChR) clusters show wider distribution in Frizled3 and Celr3

knockout mice, whereas no change was observed in Wnt4 mutant mice, potentially due to functional redundancy by other Wnts. This may be caused by the guidance defect of phrenic nerve or the defect in prepatterning of AChR clusters. Furthermore, we also observe that synatophysin signal is significantly reduced in the Celsr3 knockout mice. Taken together, these observations indicate an important function of Wnt signaling in regulating the proper development of neuromuscular junction.

Disclosures: L. Wang: None. **Y. Zou:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.05/C5

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Canadian Institutes for Health Research Operating Grant MOP-14137

Canadian Foundation of Innovation (equipment grant)

Fonds Recherches Quebec-Santé (FRQ-S, infrastructure grant) to the Groupe de recherche sur le Système Nerveux Central

FRQ-S Studentship

Title: Glial cells influence synaptic plasticity of competing nerve terminals at the mammalian neuromuscular junction

Authors: *H. DARABID[1,2], R. ROBITAILLE[1,2];
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Abstract: The precise wiring of synaptic connections is shaped by elimination of supernumerary inputs competing for the innervation of the same target cell. At the neuromuscular junction (NMJ), this competition depends on the synaptic efficacy of competing terminals which strengthens one input and weakens the others, leading to their elimination. However, little is known about the synaptic activity and plasticity of competing terminals. Moreover, the role of glial cells during synaptic competition remains ill defined despite their importance in the modulation of synaptic efficacy and plasticity at adult NMJs. Therefore, the goal of this work was to study synaptic plasticity of strong and weak terminals during synaptic competition and their interaction with perisynaptic Schwann cells (PSCs), glial cells at NMJs. We performed

intracellular recordings from dually innervated P7-8 mouse *Soleus* muscle fibres to assess synaptic activity and monitored PSC activity using confocal Ca^{2+} imaging. PSCs were loaded using single cell electroporation of the Ca^{2+} indicator Fluo-4 and the morphological dye Alexa 594. PSCs decode synaptic competition as revealed by tight relationship between the size of Ca^{2+} responses induced by the high frequency stimulation of each independent input and their synaptic strength (i.e. weak input generated smaller Ca^{2+} responses than the strong one). Interestingly, at the same NMJ, the strong input showed a long-lasting potentiation of neurotransmission while the weak one displayed only a small transient potentiation. Bath application of the $A2_A$ receptor antagonist SCH 58261 blocked the potentiation at weak and strong inputs. To determine whether the differential plasticity of competing terminals was related to PSCs Ca^{2+} responses, single PSCs were directly activated by photoactivation at 405 nm light of NP-EGTA (a caged Ca^{2+} molecule) electroporated in PSCs. A direct induction of a large Ca^{2+} response (mimicking activation by strong inputs) resulted in a long-lasting potentiation of the strong input while the weak terminal displayed only a transient one. However, an induction of a small Ca^{2+} response did not affect the synaptic activity of the competing terminals. Finally, photoactivation of Diazo-2 (photoactivable BAPTA) blocked PSCs Ca^{2+}-responses and altered synaptic plasticity.
Altogether, these data indicate that glial cells decode synaptic competition at NMJs and govern synaptic plasticity of competing nerve terminals. The differential plasticity of strong and weak inputs likely reflects changes in synaptic efficacy of competing terminals which could influence the outcome of synaptic competition and elimination.

Disclosures: H. Darabid: None. **R. Robitaille:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.06/C6

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CIHR, grant # 67212

Title: Postsynaptic activity is crucial for synapse elimination

Authors: Y. CHONG, *E. J. COOPER;
McGill Univ., Montreal, QC, Canada

Abstract: During development, as many neural circuits become established, the afferent inputs generally refine their connections through a process of synapse competition and elimination. For

synapse elimination to occur, the competing presynaptic axons must fire asynchronously. However, the role for postsynaptic activity in this process is less clear. It is frequently assumed that co-incident pre- and postsynaptic activity stabilizes synapses; yet we showed recently that silent synapses are not eliminated but can persist for months. This prompted us to ask whether postsynaptic activity is necessary for competition and synapse elimination.
To address this issue, we have extended our investigations on synaptic connections between preganglionic neurons and postganglionic sympathetic neurons in the superior cervical ganglion. We use mice with a deletion in the α3 nicotinic ACh receptor (nAChR) subunit gene because sympathetic ganglia in these α3 KO mice have no synaptic transmission and postganglionic sympathetic neurons have no EPSPs. None the less, the preganglionic axons in α3 KO mice form morphologically normal, but electrophysiologically silent synapses on sympathetic neurons; these silent synapses persist for at least 2-3 months (as long as we examined them). To determine how preganglionic axons compete for sympathetic neurons when postsynaptic activity is absent, we labeled presynaptic axons and postsynaptic neurons with lipophilic dyes, immunostained for pre- and postsynaptic markers, and used confocal microscopy to image connections.
In 1 month old WT mice, a single preganglionic axon established several contacts along the dendrites of a single sympathetic neuron. In contrast, in 1 month old α3 KO mice, we observed several preganglionic axons converging and circling the soma of individual α3 KO sympathetic neurons. These morphological results support our electrophysiological data: in WT mice, the number of preganglionic axons functionally innervating a sympathetic neuron was reduced from ~7-9 to ~2-4 over the first postnatal month. On the other hand, in α3 KO mice at 1 month, sympathetic neurons remained innervated by ~7-9 preganglionic axons. These results demonstrate that postsynaptic activity is crucial for synapse elimination because when it is absent, competition among axons does not occur and synapses are not eliminated. Our finding suggests that the refinement of synaptic connection requires an activity-dependent retrograde signal.

Disclosures: Y. Chong: None. **E.J. Cooper:** None.

Poster

512. Synapse Formation: PNS

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.07/C7

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH/NICHD GRANT Z01HD008869

Title: Characterization of *Drosophila* neto isoforms and their role at the neuromuscular junction

Authors: *O. E. IGIESUOROBO[1], C. RAMOS[1], D. SANDSTROM[2], M. SERPE[1];
[1]Unit on Cell. Communication, Natl. Inst. of Health/ NICHD, Bethesda, MD; [2]Lab. of Mol. Biol., Natl. Inst. of Health/ NIMH, Bethesda, MD

Abstract: Synaptogenesis requires the recruitment of neurotransmitter receptors, which comprises of their assembly, trafficking, and stabilization at specific membrane locations. The *Drosophila* neuromuscular junction (NMJ) is a glutamergic synapse that shares common features with mammalian central synapses. The ionotropic glutamate receptors (iGluRs) present at the NMJ are heterotetrameric complexes composed of three essential subunits - GluRIIC, GluRIID, GluRIIE - and either GluRIIA or GluRIIB. The mechanism underlying the clustering and stabilization of iGluRs at the postsynaptic densities (PSDs) is a major question in the field. Our lab has identified and characterized the *Drosophila neto* (*neuropilin* and *tolloid*-like) as an essential gene required for clustering of both iGluRs types at the NMJ. *neto* null mutant embryos are completely paralyzed and die with no detectable iGluR clusters at their NMJ. Our previous studies revealed that Neto functions as an essential component of the iGluR complexes and is absolutely required for iGluR clustering, organization of PSDs, and synapse functionality. *neto* locus encodes for two isoforms, Neto-A and Neto-B, generated by alternative splicing. Neto-A and Neto-B isoforms share similar extracellular and transmembrane domains but contain different intracellular parts. These two isoforms are conserved in *Drosophilidae* and appear to be differentially expressed during development. We have confirmed that both isoforms are expressed in the larval body-wall muscles in *D. melanogaster*. Furthermore, either isoform could rescue the iGluRs clustering defects and embryonic lethality of *neto* null mutants. Using isoform specific mutants, rescue constructs, and electrophysiology we are investigating the role of these isoforms in synaptic clustering of iGluRs. Preliminary morphological analyses suggest that Neto-B specifically influences the synaptic accumulation of the type-A iGluR complexes. Furthermore, electrophysiological recordings in an allelic series of *neto-B* mutants revealed quantal sizes that correlate with GluRIIA levels. These findings indicate that the two Neto isoforms may have distinct and non-redundant functions in synapse assembly at the *Drosophila* NMJ.

Disclosures: O.E. Igiesuorobo: None. **C. Ramos:** None. **D. Sandstrom:** None. **M. Serpe:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.08/C8

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: National Institutes of Mental Health MH092351

Ellison Medical Foundation

Title: Systematic characterization of synaptic morphogenesis and architecture in *Drosophila* homologs of neuropsychiatric susceptibility genes

Authors: *B. KIRAGASI, D. DICKMAN;
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Abstract: Synapses are robust molecular machines for reliably transmuting electrical and chemical signals while maintaining stable nervous system function. As such, these complex cellular junctions require exquisite control of physical topology and subsynaptic architecture to permit efficient transmission as well as the modifications necessary for plasticity. New genetic, genomic, and biochemical approaches over the past few years have led to an unprecedented discovery of synaptic genes implicated in a wide range of poorly understood neuropsychiatric diseases with little understanding of their possible roles in sculpting synaptic morphogenesis and structure. We have established a collection of 212 RNA interference (RNAi) transgenes that target Drosophila homologs of synaptic genes recently implicated in a variety of neuropsychiatric diseases, including schizophrenia, autism, Fragile X Syndrome, and bipolar disorder. Using this collection, we have undertaken a systematic screen of synaptic structure and morphogenesis at the Drosophila neuromuscular junction, a model glutamatergic synapse. Target synaptic genes were knocked down both pre- and post-synaptically and analyzed using confocal microscopy following immunohistochemical labeling of presynaptic active zones and postsynaptic densities. We quantified synaptic size, bouton number, active zone numbers and densities, as well as postsynaptic receptor structures. These efforts have revealed novel genes required for specific aspects of the morphogenesis or molecular architecture on both sides of the synapse. Interestingly, several genes cluster into common signaling pathways and phenotypes associated with specific diseases. Data will be presented on some of the most unique alterations in synaptic structure observed as well as secondary analysis on gene localization and function. Together, this screen will establish a foundation to explore how multiple signaling and effector molecules are integrated to ensure reliable synaptic morphogenesis and how dysfunction in these processes may contribute to neuropsychiatric disease pathogenesis.

Disclosures: B. Kiragasi: None. **D. Dickman:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.09/C9

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant 5R01NS031651

Title: Live imaging of activity-dependent synaptic refinement in *Drosophila* embryos

Authors: F. VONHOFF, *H. S. KESHISHIAN;
MCDB Dept., Yale Univ., New Haven, CT

Abstract: The pruning of off-target contacts is a crucial mechanism for establishing precise neural networks during development. Neural activity plays an important role in synaptic refinement in various systems, including the vertebrate visual system, where low frequency (<0.01 Hz) calcium and cyclic nucleotide oscillations are required for synaptic refinement. Activity-dependent synaptic refinement also occurs at the *Drosophila* neuromuscular junction (NMJ), where oscillatory neural activity and presynaptic calcium signaling, acting via CaMKII, modulate the motoneuron's response to the chemorepellent Sema-2a, an essential mechanism for the removal of off-target contacts. We have tested an additional Ca-dependent effector, the adenylyl cyclase *rutabaga* (*rut*), and the cAMP phosphodiesterase *dunce*, for their role in synaptic refinement. *rut* and *dnc* mutants both show an elevated frequency of mis-wired, ectopic synapses at the musculature. Using genetic interaction tests we find a functional relationship between cAMP signaling, Ca-channels and semaphorin repulsion for synaptic refinement. We are testing whether cAMP levels must also oscillate for normal synaptic development. We have also analyzed the role of Ca oscillations in embryonic growth cones in vivo using the Ca reporter GCaMP5. In order to suppress movement artifacts we used mutations of the *myosin heavy chain* (*MHC*) gene to suppress muscle contractions, allowing for time-lapse imaging throughout embryonic development. *MHC* embryos show normal muscle development, innervation, and motor system maturation, albeit with fictive motor activity. *MHC* embryos show a progression from activation of single neurons, to synchronous activation of all motoneurons, with late embryos showing coordinated activation of motoneurons in adjacent segments at the same low frequency as in wild type animals. We find that Ca oscillations are observed in both native and ectopic contacts. We are testing the hypothesis that the low frequency Ca oscillations will affect filopodial behavior, possibly through a transient rise in growth cone responsiveness to Sema2a when the Ca levels are high. These results suggest a model for *in vivo* analysis of oscillatory Ca signals in motoneuron synaptic refinement during the formation of neuromuscular junctions.

Disclosures: F. Vonhoff: None. **H.S. Keshishian:** None.

Poster

512. **Synapse Formation: PNS**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 512.10/C10

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of 3-hydroxykynurenine in the ROS production induced by peroxynitrite on primary culture astrocytes

Authors: *R. LUGO[1], P. UGALDE-MUÑIZ[1], B. PINEDA[2], M. TORRES-RAMOS[2], J. PEDRAZA-CHAVERRÍ[3], C. RÍOS[1], V. PÉREZ-DE LA CRUZ[1];
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Abstract: 3-Hydroxykynurenine (3HK) is a metabolite of the kynurenine pathway (KP), which is the main route for tryptophan degradation in mammals. 3-HK is produced from hydroxilation of kynurenine by kynurenine-3-hydroxylase. This metabolite has been widely described as an endogenous cytotoxic agent since it was described as a reactive oxygen species (ROS) generator, and this action was associated with apoptotic cell death in several cell types and models (Eastman and Guilarte, 1989; Nakagami et al, 1996; Okuda et al, 1996). However, this pro-oxidant effect of 3-HK has been discussed and questioned since several evidence showing antioxidant and protective actions of this metabolite (Leipnitz et al, 2007; Backhaus et al, 2008). The aim of this work was evaluated the effect of 3-HK (50 and 100 µM) on ROS production over time (30, 60 90, 120 and 160 min), cell proliferation and cell death (24 h after incubation) in primary astrocytes cultures. Additionally, the effect on ROS production of 3-HK was tested on astrocytes, incubated them with 25 µM of peroxynitrite (ONOO-). In the first 90 min, the 3-HK has no effect on ROS formation, however ROS production was diminished under the control at late times (120 and 160 min) by both concentrations of 3-HK tested. The 3-HK has not effect compare with the control in the cell proliferation evaluated through carboxyfluorescein succinimidyl ester and the cell death determinate by anexin V/propidium iodide assay and lactate dehydrogenase activity. In parallel, both concentrations of 3-HK abolish the ROS production induced by ONOO- in astrocytes primary cultures. These data suggests, that 3-HK exerts an antioxidant action by reducing ROS production even under basal levels and non-compromising primary astrocytes cultures, consequently 3-HK offers strong protection under pro-oxidant insult induced by ONOO- in astrocytes.

Disclosures: R. Lugo: None. **P. Ugalde-Muñiz:** None. **B. Pineda:** None. **M. Torres-Ramos:** None. **J. Pedraza-Chaverrí:** None. **C. Ríos:** None. **V. Pérez-de la Cruz:** None.**Poster**

513. Synapse Formation: CNS II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.01/C11

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSF

Title: Dynamically changing expression patterns of ephrinA/EphA family members in developing hippocampus

Authors: *A. T. HADER, M. R. PLUMMER;
Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ

Abstract:
Eph receptors and their ephrin ligands are known to control cell migration, axon guidance, topographic map formation and synaptogenesis in the developing brain. Since ephrin/Eph signaling is bidirectional and based on intercellular interactions, these proteins are potential key molecular players in the development of highly precise neuronal circuits that are crucial for normal brain function. In the hippocampus, Eph receptors and ephrin ligands have been shown to guide layer-specific afferents and regulate synaptogenesis. In ephrin-A5 double knock-out mice, for example, the precision of commissural connections in the inner molecular layer of dentate gyrus is lost, while the final pattern of other commissural connections is not altered. In addition, the density of synaptic contacts is reduced in the target areas of the associative/commissural projections and mossy fibers, but entorhinal synapses don't appear to be affected (Otal et al., Neurosci, 2006). Previous work in our lab showed that ephrinA5 signaling can modulate the function of a synaptogenic molecule, brain-derived neurotrophic factor (BDNF), in rat hippocampal cultures (Bi et al., J.Neurophysiol, 2011). Recently, we have been characterizing the dynamically changing expression patterns of several members of the ephrinA/EphA family to understand better the roles played by these molecules in the specification and maturation of synaptic connectivity. Our data indicate that different EphA receptors and ephrinA ligands show cell type-specific localization patterns that change with developmental stage and appear to be linked to areas of active synapse formation. For example, we and others (Otal et al., Neurosci, 2006) have observed that expression levels of EphA3, one of the receptors for ephrinA5, wax and wane in the target areas of the entorhinal axons yet consistently increase in associative/commissural areas during first month of postnatal development. Additionally, we see that in distinct locations of the developing hippocampus, EphA3 expression generally appears to be presynaptic, suggesting that this receptor might be involved in the process of synaptogenesis. To test explicit hypotheses as to function, we plan to employ molecular tools to knock-down

expression of different ephrinA/EphA family members in a subset of mouse hippocampal neurons and use electrophysiology to assess the involvement of these proteins in coordinating the development of layer-specific connectivity in the hippocampus.

Disclosures: A.T. Hader: None. **M.R. Plummer:** None.

Poster

513. Synapse Formation: CNS II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.02/C12

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: EphA7 splice variants differentially regulate dendritic morphology and synaptogenesis during cerebral cortical development

Authors: *C. LEONARD[1], W. ATHAR[2], M.-S. VAN DER GOES[3], D. BURTON[1], M. DONOGHUE[1];
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Abstract: Intercellular communication is a critical process that regulates the development of neurons, from migration to synaptogenesis. In order to achieve the proper shape and connections, neurons must interact in a variety of ways to determine their fate. One group of molecules, the Eph receptors and their ephrin ligands, play versatile roles in development. EphA7, in particular, has been shown to influence parcellation of brain structures, retinotectal axon guidance, and topographic mapping between the cortex and thalamus. The role of EphA7 in dendritic arborization and synaptogenesis, however, remains unclear.
Our lab has demonstrated that EphA7 is required for multiple stages of neuronal development. When EphA7 is absent from neurons, dendrites can no longer avoid ligand ephrinA5, resulting in altered cell shape. Furthermore, neurons lacking EphA7 are delayed in maturation of synaptic transmission, leading to a decrease in the number of dendritic spines in adults. Therefore, EphA7 is necessary earlier in development, during growth of cellular processes, and later, during the formation and maintenance of neuronal connections. We hypothesize that the distinct roles of EphA7 are subserved by two splice variants of the receptor: a full length form which contains an intracellular tyrosine kinase domain that typically facilitates repulsive signaling between cells; and a truncated form which lacks the kinase domain and is, therefore, incapable of signaling by the same mechanism. Using in vitro and in vivo analyses of murine cortex, we find that full length EphA7 mRNA expression peaks just before birth and decreases throughout adulthood, while truncated EphA7 expression peaks postnatally and remains expressed in the adult cortex.

Gain of function with individual isoforms in wildtype primary cortical neurons produces divergent dendritic morphological changes in culture. Additionally, electrophysiological data from whole-cell recording in acute cortical slices reveal differential synaptic effects when individual isoforms are expressed in EphA7-/- neurons. Our results support a model in which the repulsive, full length EphA7 receptor acts during early neuronal development as a neuron is extending processes to connect with synaptic partners while the permissive truncated EphA7 guides the formation and function of excitatory synapses in adulthood. To our knowledge, this is the first study to investigate the distinct function of the truncated EphA7 receptor since its discovery.

Disclosures: C. Leonard: None. **W. Athar:** None. **M. van der Goes:** None. **D. Burton:** None. **M. Donoghue:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.03/C13

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: T32-MH073124 (SAS) through the UC Davis MIND Institute Autism Research Training Program

NINDS Grant R01-NS060125 (AKM)

NEI Grant R01-EY13584 (AKM)

Title: Shank dependent changes in cortical synapse dynamics during neuronal maturation

Authors: *S. A. SPANGLER, L. A. NEEDLEMAN, X.-B. LIU, B. A. MEYERS, A. MCALLISTER;
Univ. of California Davis, Davis, CA

Abstract: Mammalian central nervous system development requires the establishment of trillions of connections between neurons that form the circuits involved in learning, memory, and cognition. A large and growing list of molecules have recently been identified to influence synapse development. Generally, molecules that positively regulate synapse density are posited to affect synapse formation, while those that negatively regulate it are thought to mediate synapse elimination. However, at any given moment, synapse density is dependent on the balance of the creation of new connections and the disassembly of preexisting ones, as well as

the relative stability of each individual synapse. We have designed an in vitro long-term live imaging assay that allows us to observe, quantify, and manipulate developing cortical synapses over periods from hours to days. By expressing fluorescently tagged pre- and postsynaptic proteins in neighboring cultured neurons, we are able to identify sites of synaptic contact in live neurons and follow them over time as synapses are formed, maintained, and eliminated. Using this assay, we have observed that developing synapses are highly dynamic, with fewer than 50% of synapses persisting for more than 90 minutes at 7 days in vitro (div). Though synapses continue to be formed and eliminated into maturity, their stability increases as the neurons mature. By 10div, nearly three-quarters of synapses persist for at least an hour and a half. Shank proteins, which are known to affect synapse density, have pronounced effects on synapse dynamics as well. At 8 div, loss of Shank2 or Shank3 dramatically limits synapse stability while also diminishing both synapse formation and elimination. We are currently investigating whether these effects are similar in other stages of neuronal development, as well as examining exactly how changes in the relative rates of synapse formation and elimination result in changes in synapse density over time.

Disclosures: S.A. Spangler: None. **L.A. Needleman:** None. **X. Liu:** None. **B.A. Meyers:** None. **A. McAllister:** None.

Poster

513. Synapse Formation: CNS II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.04/C14

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Veteran's Affairs Grant

Miriam and Sheldon Adelson Foundation on Neural Repair and Rehabilitation

HHMI

Life Sciences Research Foundation

Title: Semaphorin5a-plexina2 signaling inhibits synaptogenesis in hippocampal dentate granule neurons

Authors: *Y. DUAN[1], S.-H. WANG[2], J. SONG[3], Y. MIRONOVA[1], H. SONG[4], A. L. KOLODKIN[2], R. J. GIGER[1];
[1]Cell and Developmental Biol., Univ. of Michigan, ANN ARBOR, MI; [2]Solomon H. Snyder

Dept. of Neurosci., The Johns Hopkins Univ. Sch. of Medicine, HHMI, Baltimore, MD; [3]Solomon H. Snyder Dept. of Neurosci., [4]Solomon H. Snyder Dept. of Neuroscience, Dept. of Neurol., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Autism is a highly heritable developmental brain disorder. Mounting evidence suggests that altered synaptic connectivity and function are major causes of autism spectrum disorders (ASDs). A genome-wide-association study identified 3 SNPs near the human *Semaphorin5A* (*SEMA5A*) locus and reported a reduced *SEMA5A* expression in the occipital lobe of autistic brains (Weiss et al., 2009), suggesting that *SEMA5A* is a novel candidate ASD susceptibility gene. Sema5A and its close relative Sema5B are transmembrane proteins characterized by the presence in their ectodomains of a sema domain and a tandem array of seven canonical thrombospondin-type 1 repeats (TSRs). *Sema5A* and *Sema5B* show overlapping, yet distinct, expression patterns in the developing mouse brain and are present in the hippocampus throughout adulthood. To investigate the role played by neuronal *Sema5A*, we analyzed synapse development in the hippocampus using mouse genetics. Ablation of *Sema5A*, but not *Sema5B*, leads to a significant increase in dendritic spine density in both developmentally-born and adult-born dentate granule cells (GCs). Electrophysiological study reveals a significant increase in mEPSC amplitude, but not frequency, in *Sema5A*$^{-/-}$ GCs. At day 21 in culture, *Sema5A*$^{-/-}$ GCs exhibit a greater dendritic spine density than wild type GCs. Expression of recombinant Sema5A rescues the increase in spine number observed in *Sema5A*$^{-/-}$ GC cell-autonomously. We also identified a high-affinity interaction between Sema5A and PlexinA2 *in vitro*. *In situ* hybridization and biochemical data show that PlexinA2 is expressed robustly in the mouse hippocampus. Further, Sema5A and PlexinA2 are both enriched in synaptosomal fractions isolated from the mouse hippocampus. Similar to *Sema5A*$^{-/-}$ mutant mice, *PlexinA2*$^{-/-}$ mice exhibit a significant increase in GC spine density. Furthermore, our functional studies performed in primary GCs show that Sema5A-mediated regulation of dendritic spine density is *PlexinA2*-dependent. The PlexinA2 cytoplasmic domain is necessary for the spine density reduction. Finally, behavioral analysis using the three-chamber test allowed us to identify abnormal social interaction in *Sema5A*$^{-/-}$ congenic mice, suggesting that loss of *Sema5A* leads to a deficit in communication skills, a hallmark of ASD. Therefore, our results support the idea that defects in neuronal cell morphology and synaptogenesis contribute to ASD endophenotypes, identifying a specific guidance cue signaling pathway that may be critical for normal acquisition of social interaction behaviors.

Disclosures: Y. Duan: None. **S. Wang:** None. **J. Song:** None. **Y. Mironova:** None. **H. Song:** None. **A.L. Kolodkin:** None. **R.J. Giger:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.05/C15

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH RO1 GM066897

T32 GM007491

Title: Searching for synaptic connectivity molecules using the *C. elegans* male

Authors: *M. I. LAZARO-PENA, B. KIM, S. W. EMMONS;
Albert Einstein Col. of Med., Bronx, NY

Abstract: How neurons recognize their correct synaptic partners remains largely an unsolved problem. Additional synapse-level connectivity from the new field of connectomics emphasizes the complexity of this process. Our studies of the C. elegans male have defined connectivity among neurons in a neural network that governs mating behavior. The 185 neurons in the network are extensively connected to each other, over a hundred-fold range of strengths, by over 10,000 chemical and electrical synapses. Each neuron makes synapses with many other neurons (average 15-20). Every neuron interacts strongly with some of its partners and weakly with others, forming a smooth distribution from strong to weak. Synaptic partners are similar for equivalent neurons, including the weakly-connected partners—weak connections carry a significant fraction of the load through the network. This indicates that even the weak set of interactions are not random. As male mating is an innate behavior, we conclude that both synaptic partners and strength of interaction are genetically specified. Despite decades of mutational analysis of behavior in C. elegans aimed at dissecting nervous system development and function, only a few genes involved in synaptic connectivity have so far been identified, a seemingly inadequate set to explain the complexity of the male mating network. Therefore, in order to identify additional synapse-specificity molecules, we are taking multiple approaches. Using cell-specific reporter genes that allow us to visualize complex patterns of synapses, we will test the function of candidate genes, blocking their expression by mutation or RNAi. Second, by comparing the transcriptomes of different classes of neurons in single cell-type transcriptome analysis, we hope to identify genes whose expression pattern correlates with particular patterns of connectivity. Third, since the male generates a large number of synapses during a short interval at the end of larval development, we are constructing a time series of the whole-animal transcriptome to identify a set of genes co-expressed at higher levels during this interval. We have shown that increased expression of genes for known synaptic components can be detected at this time and anticipate that still unknown genes for synaptogenesis can be identified by this signature. Finally, we will use reporter genes to compare patterns of connectivity in a set of wild C. elegans strains in hopes of identifying natural variants that can be genetically mapped.

Disclosures: M.I. Lazaro-Pena: None. **B. Kim:** None. **S.W. Emmons:** None.

Poster

513. Synapse Formation: CNS II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.06/C16

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: A new automated synaptoneurosome preparation: Comparison between traditional and new techniques

Authors: *J. L. BALSOR[1], K. M. MURPHY[2];
[2]McMaster Integrative Neurosci. Discovery and Study, [1]McMaster Univ., Hamilton, ON, Canada

Abstract: The synaptoneurosome preparation is a popular subcellular fractionation technique that enriches synaptic components and has become a valuable tool for studying synaptic proteins. The traditional technique, developed almost 30 years ago, has not been validated across development or updated to include modern technology. The immature nature of early postnatal cortex, especially in rodents where neurons are still migrating into the cortical plate, growing, and differentiating, raises questions about the consistency of the preparation across ages. We became concerned that the hand homogenization and filtration steps may introduce variability because of the structural immaturity of early postnatal cortex. To address this concern, we compared the traditional hand homogenization and syringe filtration technique with a new technique that we developed using automated tissue homogenization (FastPrep-24, MP Biomedicals) and centrifugal filtration (Ultrafree-MC Centrifugal Filter Tubes, Millipore). We then compared the traditional synaptoneurosome preparation with our new preparation by using Western blots to compare enrichment of synaptic proteins in immature and mature rat cortex. We used synaptic proteins: PSD95 -- a scaffolding protein that anchors glutamate receptors; Gephyrin -- a scaffolding protein that anchors GABA receptors, Synapsin --a marker for pre-synaptic terminals; and Synaptophysin -- a membrane protein on synaptic vesicles. Enrichment was calculated as the ratio between protein expression in synaptoneurosome and homogenate preparations. The updated preparation agreed with previous studies for mature tissue samples, showing consistent two to threefold enrichment. The traditional method produced highly variable enrichment in tissue samples taken from immature cortex. Our new method substantially reduced that variability. These results show the traditional synaptoneurosome preparation is highly variable in early postnatal cortical tissue and could bias interpretation of results. In contrast, our new automated synaptoneurosome preparation produces a consistent level of synaptic protein enrichment in both immature and mature cortical tissue samples.

Disclosures: J.L. Balsor: None. **K.M. Murphy:** F. Consulting Fees (e.g., advisory boards); Allergan.

Poster

513. Synapse Formation: CNS II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.07/C17

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH R01 DC007695

NIH/NIGMS CoBRE P20 GM103503

NIH/NIGMS P41 GM103412

Title: Segregated innervation and nuclear territories in a polarized cell body during development of the calyx of Held

Authors: *P. S. HOLCOMB[1], M. HOYSON[1], D. R. JACKSON[1], K. MOTWANI[1], B. M. KELLERMEYER[1], M. H. ELLISMAN[2], T. J. DEERINCK[2], G. A. SPIROU[1];
[1]Sensory Neurosci. Res. Ctr., West Virginia Univ. Sch. of Med., Morgantown, WV; [2]Natl. Ctr. for Microscopy and Imaging Res., UCSD, San Diego, CA

Abstract: Cell polarity--both in terms of morphological structure and location of intracellular organelles--plays a vital role in the correct differentiation, migration, and function of eukaryotic cells. In the CNS, defects in proteins vital for the establishment and maintenance of polarity have been implicated in a host of developmental disorders. However, little is known about the role of neuron polarity in synaptogenesis, which may have implications in disorders linked to miswiring such as schizophrenia and autism. To examine the relationship between cell polarity and innervation, we utilized serial block-face scanning electron microscopy (SBEM) and manual three-dimensional reconstruction of the developing calyx of Held and its postsynaptic partner, the principal cell of the medial nucleus of the trapezoid body (MNTB), in the mouse auditory brainstem. This system is ideal for studying the dynamics of synaptogenesis due to the rapid growth dynamics of the calyx (24-48 hours) and a well-defined endpoint of mono-innervation, with each principal cell receiving only a single calyceal input. In order to define nuclear eccentricity, the center of mass (or centroid) of the principal cell and its nucleus were calculated. A plane perpendicular to the vector between these two points and passing through the cell body centroid was used to define distinct somatic “poles”, and the volume of the nucleus contained within each pole was calculated. Similarly, the surface area of the growing calyceal terminal

residing within each pole was determined. This analysis revealed eccentricity in the positioning of the nucleus within the principal cell body, which is present as early as postnatal day 2 (P2) and persists until at least P6. Additionally, preliminary analysis suggests that innervation of the principal cell by potential calyx-forming axons occurs on the somatic surface not containing the nucleus. 3D reconstructions also show a significant wrapping of the principal cell surface by glia at P2, which is removed by P3 preferentially on the non-nuclear pole of the cell. We propose that the position of the nucleus eccentrically within the principal cell body and lack of innervation on this "nuclear pole" is indicative of a polarity-mediated program coordinated between neurons and glia for defining innervation territory for the growing calyceal input. This polarity program may generalize to other model systems in mammals for synaptogenesis during early brain development.

Disclosures: P.S. Holcomb: None. **M. Hoyson:** None. **D.R. Jackson:** None. **K. Motwani:** None. **B.M. Kellermeyer:** None. **M.H. Ellisman:** None. **T.J. Deerinck:** None. **G.A. Spirou:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.08/C18

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CONACyT grant No. 104659

CONACyT grant No. 101316

Title: The reduction of latency of action of antidepressants by 17β- estradiol is related to the stimulation of neuronal maturation

Authors: *N. VEGA[1,3], J. FERNÁNDEZ[4], G. RAMÍREZ[2], E. ESTRADA[2];
[1]1Laboratory of Neuropsychopharm., [2]Neuropsychopharm., Natl. Inst. of Psychiatry, México DF, Mexico; [3]Departament of Pharmacobiology, [4]2Departament of Pharmacobiology, Ctr. for Res. and Advanced Studies (CINVESTAV), México D.F., Mexico

Abstract: Several reports indicate that estrogens potentiate the effects of antidepressants and facilitate the action of these drugs by shortening their latency of onset. Estrogens also have a variety of actions on the central nervous system such as the regulation of neuronal excitability, the increase in birth of new cells via the neurogenic process and the synapse formation. Thus, the aim of the present work was to explore if the antidepressant-like effect induced by the

combination of sub-optimal doses of estradiol plus fluoxetine involves changes on cell proliferation, early survival and maturation.
The antidepressant-like effect of the combination of sub-optimal doses of fluoxetine (FLX)-estradiol (E_2) was evaluated in ovariectomized Wistar rats exposed to the forced swimming test (FST, an animal model for the screening of antidepressant drugs). To study the effects of the combination FLX-E_2 on cell proliferation (determined by endogenous marker Ki67), the survival of newborn cells (established with the thymidine analog BrdU; 75 mg/kg, 2/12, i.p before treatment) and on dendrite organization cells (determined by endogenous marker doublecortin), 60 min after the FST the animals were perfused and the brain was dissected. Animals were assigned to one of the following treatments: Vehicle (saline/14 days + Oil/-8h before FST); E_2 (saline/14 days + 10 µg / rat; -8h before of test); FLX (fluoxetine; 5mg/kg/ 14 days + oil/ -8 h before FST), E_2 (saline/14 days + 2.5µg / rat; -8 h before of test); FLX (1.25 mg / kg / 14 days + oil/-8h before to FST) and FLX/E2 (1.25 mg / kg / 14 days+/-8h before to FST).
Results showed that non-effective doses of FLX or E2 given independently did not produce changes on FST or in the number of positive immunoreactivty cells to Ki67, BrdU or doublecortin. In contrast, FLX at dose of 10 mg/kg and estradiol at dose of 10 µg/rat produced an antidepressant-like action in the FST. However, despite of both treatments increased the neuronal maturation, only the FLX also produced changes on cell proliferation and survival. The combination of FLX and E2 at non-effective dose produced an antidepressant-like effect in the forced swimming test and increased the number of new neurons, identified by doublecortin expression, in last phases of dendrite maturation but did not induce changes on cell proliferation or survival.

Disclosures: N. Vega: None. **E. Estrada:** None. **G. Ramírez:** None. **J. Fernández:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.09/C19

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH (RO1-NS064014)

UW Madison Vilas Award

Title: The role of dynamic microtubules in stabilization of estradiol-induced dendritic spines

Authors: *M. MILLETTE, K. TAYLOR, E. W. DENT;
Neurosci., Univ. of Wisconsin - Madison, Madison, WI

Abstract: Dendritic spines are the major sites of excitatory synaptic transmission in the brain and are widely considered an anatomical substrate for learning and memory. During early development there is a surge in formation and incredible turnover of dendritic spines as functional connections are made with other neurons. In-vivo studies illustrate that many spines may remain stable once formed, but formation and elimination of spines continues throughout adult life. Numerous factors contribute to this plasticity. Notably, estradiol application to cultured neurons is known to increase spine density on a timescale of minutes. Preliminary evidence suggests that estradiol-induced spines are far more transient in nature than previously believed. Using multi-wavelength total internal reflectance fluorescence microscopy (TIRFM) we show a rapid increase in the number of transient dendritic protrusions following estradiol application to mature cortical neurons, but a subsequent disappearance of most protrusions within seconds. Published evidence suggests NMDA receptor activation is involved in stabilizing these estradiol-induced protrusions and estradiol application results in increased PSD-95 at synapse. Our lab has previously shown that microtubules (MTs) directly enter dendritic spines through bouts of dynamic polymerization. We have also shown that MT-invaded spines enlarge in an NMDA receptor- and MT-dependent manner and that synaptic PSD-95 expression following BDNF treatment is dependent on this MT invasion. Therefore, we are currently manipulating MT dynamics and chemically inducing LTP post-E2 in order to probe the role of dynamic MTs in estradiol-induced spine generation and permanency.

Disclosures: M. Millette: None. **K. Taylor:** None. **E.W. Dent:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.10/C20

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Young Investigator Grant 18741 (S.L.B)

NIH R01-EY 13584 (A.K.M)

Title: Opposing roles of glutamate and neuroligin/neurexin adhesion in cortical synapse formation

Authors: S. L. BARROW[1], *A. MCALLISTER[2];
[1]Ctr. for Neurosci., UC Davis, Davis, CA; [2]Ctr. for Neurosci., UC Davis, DAVIS, CA

Abstract: Formation of synaptic connections is integral to the establishment of the complex circuitry underlying perception, cognition, and behavior. Recruitment of glutamate receptors to nascent synapses is critical to this process. Transport packets rapidly deliver NMDA receptors (NMDARs) to newly forming synapses following initial axo-dendritic contact (Washbourne et al., 2002) via a neuroligin (Nlg)-dependent mechanism (Barrow et al., 2009). Their transport along dendrites occurs bi-directionally , interspersed with frequent pauses at sites where NMDARs cycle with the plasma membrane (Washbourne et al., 2004). Periodic delivery to the dendritic surface raises the possibility that NMDARs are capable of sensing glutamate during their transport, enabling local changes in glutamate secretion to alter their recruitment to newly forming synapses. However, it has been thought that glutamatergic transmission plays no role in synapse formation since synapses can clearly form in the absence of neurotransmitter release (Verhage et al., 2000, Varoqueaux et al., 2002). Using a combination of imaging, electrophysiology and genetic approaches in cultured visual cortical neurons, we sought to determine the role of glutamate and NMDAR activation during the initial establishment of connectivity. Acute pharmacological activation of NMDARs and stimulation with glutamate, globally and locally, decreases the mobility and surface expression of NMDARs, the proportion of mobile NMDARs that are transported with Nlg, and the density of synapses formed. NMDAR blockade has the reverse effect. Moreover, the glutamate-induced decrease in NMDAR transport is dependent on NMDAR-mediated Ca2+ influx. Most important, these effects of glutamate and NMDAR activation are prevented specifically at sites of association of the NMDAR/Nlg complex with neurexin in a mixed co-culture assay. Taken together, these results suggest that glutamate and NMDAR activation play an important role in determining the number of glutamatergic synapses on cortical neurons. Glutamate release appears to regulate synapse formation by destabilizing postsynaptic components that fail to make contact with presynaptic partners, similar to the opposing roles for ACh and agrin at the NMJ.

Disclosures: S.L. Barrow: None. **A. McAllister:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.11/C21

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NINDS F32 NS074839

NINDS R21 NS076950

Title: Molecular mechanisms of electrical synapse development

Authors: *A. MILLER, L. VOELKER, C. MOENS;
Inst. of Neurosci., Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract: Neural circuits, particularly the patterns and properties of their synapses, underlie all behavior. Synapses can either be chemical, where signals are transmitted via neurotransmitter release and reception, or electrical, where signals pass directly through gap junctions between neurons. In order to make circuits, neurons must direct their processes to the correct location, recognize their synaptic partners, and transport proteins to the sites of synaptogenesis. While a number of pathways have been identified that regulate chemical synaptogenesis, the genes that regulate electrical synapse formation are largely unknown.
We are using the zebrafish Mauthner (M) circuit as a model to address electrical synapse formation. The M circuit is made up of individually identified neurons that communicate at unique, stereotyped synapses to generate a fast avoidance turn in response to threatening stimuli. The M neuron is a large interneuron whose cell body is in the hindbrain and whose axon extends contralaterally down the spinal cord. Presynaptic sensory neurons in the ear and lateral line make mixed chemical/electrical synapses on the M cell body and dendrites, and M in turn makes chemical synapses with primary motor neurons and electrical synapses with inhibitory CoLo interneurons in the spinal cord. A forward genetic screen looking for mutations that disrupt M electrical synapses identified two classes of mutations: Disconnect (Dis, 3 alleles) in which electrical synapses are reduced or absent and Amped (Amp, 2 alleles) in which supernumerary electrical synapses form. These phenotypes suggest that there are positive (Dis genes) and negative (Amp genes) regulators of electrical synapse formation.
To identify the genes disrupted in the mutants we have employed a novel RNA-seq-based mapping pipeline and have found a nonsense mutation in the autism-associated gene neurobeachin (nbea) in Dis4 mutants. A TALEN induced lesion in nbea does not complement Dis4 confirming that nbea is causal for the electrical synapse defect. Nbea is a highly conserved, multidomain protein that is implicated in synaptic protein trafficking in neurons. Using chimeric analysis in which a limited number of cells are mutant in an otherwise wildtype animal we have found that Nbea is required in the postsynaptic neuron for electrical synapse formation. Our findings suggest that Nbea regulates the transport of proteins necessary for postsynaptic, but not presynaptic, electrical synapse formation.

Disclosures: A. Miller: None. **C. Moens:** None. **L. Voelker:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.12/C22-DP1

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: European Molecular Biology Organization (EMBO)

Max-Planck Society Germany

NIH

HHMI

Title: Crystal structures of the C1q-like protein family reveal conserved Ca2+ binding motifs crucial for BAI3 GPCR interaction and synapse homeostasis

Authors: *S. RESSL[1], D. C. MARTINELLI[1], B. K. VU[1], T. C. SÜDHOF[1,2], A. T. BRUNGER[1,2];
[1]Stanford Univ., Stanford, CA; [2]HHMI, Stanford, CA

Abstract: Background C1q-like (C1ql) are secreted proteins of yet unknown function belonging to the C1q/TNF super-family. Recent findings show that a) C1ql proteins are expressed in neurons (1) and have Ca^{2+} dependent affinity for the brain-specific angiogenesis inhibitor 3 (BAI3) G-protein coupled receptor (GPCR) (2). These findings suggest a role for C1ql in synapse homeostasis. Here we describe the crystal structure of the globular C1q domains of C1ql1-3, and residues involved in Ca^{2+}-dependent binding to BAI3. We characterize the specific structural and functional differences within the C1ql family and propose a functional role as specific regulators in synapse homeostasis via Ca^{2+}-dependent binding to BAI3. **Methods** We used X-ray crystallography to determine the crystal structures of the C1ql protein family. Based on the structures, we mutated a series of specific ion binding residues. The structural integrity and stability of purified C1ql proteins and mutants was verified by CD-spectroscopy. We measured the Ca^{2+} binding affinity of C1ql proteins and mutants to elucidate differences in ion binding observed in the structures using isothermal calorimetry. Fluorescence labeled cell-surface binding assays and biolayer interferometry were used to characterize C1ql and mutants binding to BAI3. Furthermore we employed neuronal culture experiments to study C1ql and mutants effect on synapse number and dendrite growth in cultured neurons. **Results** The crystal structures of C1ql proteins reveal structural and ion-binding differences within the family and reveal conserved Ca^{2+} binding motifs essential for BAI3 receptor binding. Whereas the crystal structures of C1ql1 and C1ql3 show ion-binding regions, the structure of C1ql2 does not. A specific arginine residue conserved in C1ql2 across species replaces positive charged ions observed in C1ql1 and C1ql3 at the identified Ca^{2+}-dependent BAI3 binding site. Because C1ql2 and C1ql3 can form hetero-trimers in the brain (1), the difference in ion binding properties of C1ql proteins suggests a regulatory element of receptor binding specificity in dependence of Ca^{2+} ion concentration. C1ql3 mutants show decreased structural stability and a loss in BAI3

receptor binding. C1ql3 mutants also vary from wildtype C1ql3 in their effect on synapse homeostasis in cultured neurons. **Conclusions** The presence of the Ca^{2+} binding motifs is crucial for BAI3 receptor binding. We suggest that C1ql proteins play an important regulatory role in synapse homeostasis in specific regions of the brain by binding to BAI3.

Disclosures: S. Ressl: None. **D.C. Martinelli:** None. **B.K. Vu:** None. **T.C. Südhof:** None. **A.T. Brunger:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.13/C23

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: PirB is a receptor for LGI1 and alters seizure progression in mice

Authors: *R. A. THOMAS[1], C. X. Q. CHEN[1], V. SOUBANNIER[1], S. BAULAC[2], P. A. BARKER[1];
[1]CNS, Montreal Neurolog. Inst. McGill Univ., Montreal, QC, Canada; [2]CRICM UPMC, Hôpital de la Pitié-Salpêtrière, Paris, France

Abstract: LGI1 is the first non-ion channel protein to be identified as causative in epilepsy in humans: a mutation in a single allele results in autosomal dominant lateral temporal lobe epilepsy. We previously identified LGI1 as a novel ligand for Nogo Receptor 1 (NgR1), a GPI-linked protein that was initially identified as a receptor for myelin-derived neuronal growth inhibitors (MGIs). NgR1 has also been shown to restrict synaptic plasticity during critical period closure and memory retention in the CNS. A structurally unrelated receptor, termed PirB, has likewise been implicated in restricting activity-dependent synaptic plasticity as well as functioning as a receptor for MGIs.
Intriguingly, we have recently found that PirB is a high affinity receptor for LGI1 and demonstrated that NgR1 and PirB are associated in a cell surface complex when expressed in heterologous cells. Identification of this receptor complex led us to ask if NgR1 and PirB have a role a in seizure progression in mice. Complete loss of LGI1 function in mice results in spontaneous seizure activity and death, LGI1-/+ mice do not have spontaneous seizures but do have a reduced threshold for seizure induction. We tested whether mice rendered null for NgR1 or for PirB, have a reduced threshold for seizure induction and found that deletion of either gene results a reduced seizure threshold.
Our results indicate that NgR1, PirB and LGI1 could interact in the formation of neural circuitry

involved in seizure progression. Recently, both NgR1 and LGI1 have been shown to regulate synaptic refinement in different contexts and we are currently investigating the effect of LGI1 and NgR1 on synapse formation and maintenance within central nervous system neurons.

Disclosures: R.A. Thomas: None. **C.X.Q. Chen:** None. **V. Soubannier:** None. **P.A. Barker:** None. **S. Baulac:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.14/C24

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH/NINDS RO1NS065856

Title: Sema4D signaling in mammalian CNS GABAergic synapse development

Authors: *A. R. MOORE, M. S. KUZIRIAN, A. J. RASSI, S. PARADIS;
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Abstract: While there are a number of molecules known to mediate glutamatergic synapse development, relatively few molecules have been identified as mediators of GABAergic synapse development. We have previously identified the transmembrane protein, Semaphorin4D (Sema4D) as an important regulator of GABAergic synapse formation, using a forward genetic RNAi-based screen. Semaphorins are a family of proteins that are implicated in a number of cellular processes including axon guidance, T cell activation, angiogenesis and recently, synapse development. However, the exact function of Sema4D and the receptor through which it signals to regulate GABAergic synapse formation remains unknown. To address this question, we first constructed multiple chimeras of Sema4D, including a non-cleavable form, by replacing different domains of Sema4D with that of the transmembrane protein CD4, a small single pass protein involved in T-cell activation. This approach revealed that Sema4D signals through its extracellular domain as a membrane-bound protein localized to the synaptic membrane in mammalian hippocampus. From this result, we then went on to examine the function of Sema4D in mediating GABAergic synapse formation by utilizing a combination of immunocytochemistry and electrophysiology in cultured hippocampal neurons and acute hippocampal slice treated with the functional, extracellular Sema domain from Sema4D. We found that treatment of cultured neurons with the extracellular domain of Sema4D causes a rapid increase (i.e. 30 minutes) in GABAergic synapse density (as assayed by immunocytochemistry) accompanied by an increase in miniature inhibitory postsynaptic current frequency within 2 hours (as assayed by whole-cell

voltage clamp recordings). Importantly, Sema4D treatment was unable to drive GABAergic synapse development when these experiments were performed on neurons isolated from mice in which the putative Sema4D receptor PlexinB1 had been constitutively deleted. These data strongly suggest the Sema4D is capable of rapidly initiating functional GABAergic synapse development in a PlexinB1-dependent manner.

Disclosures: A.R. Moore: None. **M.S. Kuzirian:** None. **A.J. Rassi:** None. **S. Paradis:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.15/C25

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: DFG through the CRC889

Title: A point mutation abolishes presynaptic targeting of the synaptic vesicle protein mover

Authors: A. AKULA, F. WETZEL, A. PETKOVA, J. HOEBER, T. GHELANI, N. WITTENMAYER, *T. DRESBACH;
Univ. of Goettingen, Goettingen, Germany

Abstract: Synapses are asymmetric cell-cell contacts.Targeting of synaptic vesicles to presynaptic sites is one of the most intricate examples of polarized trafficking and site-specific accumulation. Suprisingly little is known about amino acid sequences or strucutral requirements mediating presynaptic targeting of synaptic vesicles proteins. Here we have characterized presynaptic targeting of Mover / TPRGL / SVAP30, a 266 amino acid protein associated with synaptic vesicles as a peripheral membrane protein. We used expression of GFP-tagged versions of Mover in cultured hippocampal neurons as a screening system, testing which recombinant variants of Mover undergo presynaptic targeting. We found that deleting either of 4 predicted phosphorylation sites did not impair targeting. By contrast, introducing a phenylalanine to arginine mutation into a c-terminal area of Mover completely abolished presynaptic targeting, leading to a diffuse distribution of the mutant protein. To delineate the domain mediating targeting we expressed several deletion constructs. However, neither an n-terminal region, a c-terminal region nor a central region allowed for targeting. These deletion constructs also failed to dimerize, as revealed in co-immunoprecipitation assays. A yeast-2-hybrid assay, and co-immunoprecipitation corroborated the strong tendency of Mover to undergo homomeric interaction. These data suggest that Mover forms dimers or oligomers as a prerequisite for a

phenylalanine residue to become exposed and mediate association with components of the axonal trafficking machinery.

Disclosures: A. Akula: None. **T. Dresbach:** None. **F. Wetzel:** None. **A. Petkova:** None. **N. Wittenmayer:** None. **J. Hoeber:** None. **T. Ghelani:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.16/C26

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant EY021865

Title: The Wrb gene encodes a novel protein required for ribbon synapse function

Authors: *L. L. DANIELE[1], F. EMRAN[2], B. PERKINS[3];
[1]Ophthalmic Res., Cole Eye Inst., Cleveland, OH; [2]Ctr. for Res. in Neurosci., McGill Univ., Montreal, QC, Canada; [3]Ophthalmic Res., Cole Eye Institute, Cleveland Clin., Cleveland, OH

Abstract: Ribbon synapses of sensory neurons tonically release glutamate to signal graded changes in stimulus intensity. The ribbon structure tethers numerous synaptic vesicles in close proximity to the presynaptic active zone, and is composed mainly of the scaffolding protein ribeye. The zebrafish *hi1482* mutant was isolated with defects in vision and mechanosensory signaling. The mutation results from a retroviral insertion in a gene encoding the novel protein, tryptophan rich basic protein (Wrb).The objective of this study was to investigate the *wrb* mutant to gain insight into the role of this novel protein in ribbon synapse structure and function. Electroretinography (ERG), Immunohistochemistry (IHC) and electron microscopy (EM) analyses were employed to assess ribbon synapse function, protein expression, and ultrastructure. w*rb* mutants were crossed with transgenic fish expressing membrane-tethered YFP in ON bipolar cells, Tg(nyx:mYFP), to investigate the morphology of ON bipolar cell contacts. *wrb* mutants at 5 dpf had diminished cone - derived ERG b-waves, which arise from ON bipolar cells. Cone derived a-waves had normal amplitudes. The mechanosensory hair cells of the lateral line organ had severely decreased FM143 staining, consistent with a defect in vesicle release. Ribeye localization was disrupted in *wrb* mutants, having a diffuse distribution pattern at synapses of photoreceptors and lateral line hair cells. EM analysis revealed a decrease in the number of presynaptic ribbons, with ribbons frequently found floating in the synaptic cytoplasm. Synaptic vesicle protein 2 (SV2) was also mislocalized, consistent with evidence of disrupted ribbon assembly. Preliminary evidence also suggested disrupted expression of the presynaptic

calcium channel subunit, CaV1.3a, in hair cell synapses. Despite disrupted ribbon synapse structure, the post-synaptic contacts of bipolar cells and horizontal cells at cone synaptic terminals were structurally normal in *wrb* mutants, with no aberrant contacts detected in YFP labeled bipolar cells.
The attenuated synaptic transmission at ribbon synapses and the mislocalization of key presynaptic components in the *wrb* mutant suggests that Wrb is important for the assembly or maintenance of ribbon synapses. Interestingly, retinal cone photoreceptors maintained appropriate contacts with post-synaptic neurons, despite the reduced transmission in the mutants, suggesting that the maintenance of synaptic contacts may not depend on synaptic release, or that residual synaptic function is sufficient.

Disclosures: L.L. Daniele: None. **F. Emran:** None. **B. Perkins:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.17/C27

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: KAKENHI 23300141

Title: Slow, stable, and long-distance retrograde movement of synaptophysin-positive puncta along axons in corticospinal slice coculture

Authors: *N. YOSHIOKA, N. ISOO, N. MURABE, H. KAMEDA, I. TAKAHASHI, M. SAKURAI;
Dept. Physiol., Teikyo Univ. Sch. Med., Tokyo, Japan

Abstract: Synapse formation requires transport of presynaptic components along the axons to the target area. Synaptic vesicles are transported and accumulated at presynaptic sites. In organotypic corticospinal slice coculture system, we used EGFP-tagged synaptophysin (Syp-EGFP) as a marker for presynaptic vesicles and tried to capture the whole sequence of events that proceed along the axons in the target tissue. By way of in vitro electroporation, we labeled cortical cells with Syp-EGFP and monitored the dynamic properties of the distribution of the protein along the axons. We also introduced DsRed2 to visualize axonal morphological change at the same time. To perform time-lapse imaging of the fluorescence signals from those corticospinal axons in static slice coculture, we developed a system using a custom made stage top CO_2 incubator equipped to a non-inverted confocal microscope stage. This CO_2 incubator can take a dry objective lens without having condensation. This system allows us to image

fluorescence signals repeatedly at the interval of 5-60 min for more than 10 hours under ordinary static culture condition for brain slices kept on a membrane culture insert. We found that, at already 2 days in vitro (2 DIV), Syp-EGFP signal was accumulated to form puncta. Those puncta were associated with axonal swellings or varicosities visualized by DsRed2. Unexpectedly, when time-lapse image acquisitions were made at an interval of 60 min, those puncta showed slow (10-50 μm/hour, a hundred times slower than the rate previously reported), continuous, stable and long-distance (sometimes exceeding 300 μm) movement along the axons. Puncta movements coincided with varicosity movements and were dominantly retrograde at all the culturing days examined (2-7 DIV). In cases movements lasted for more than 3 hours at a constant rate, they were sometimes interrupted by elongated pauses for more than 1 hour. The movements were also monitored at 5 min intervals and rates of constant movement comparable to that monitored at 60 min intervals were obtained, which further supported continuous and stable movement. The frequency of puncta to show movement in 6 hours was 30 % at 3 DIV and declined gradually until 7 DIV with a frequency of less than 10 %. As the time point of 7 DIV coincides with that of synaptogenesis in the corticospinal slice coculture, we hypothesized that those migrating puncta are a type of prepared packages for presynaptic apparatus, searching for potential synaptic sites and cease their movement when captured by those sites.

Disclosures: N. Yoshioka: None. **N. Isoo:** None. **N. Murabe:** None. **H. Kameda:** None. **I. Takahashi:** None. **M. Sakurai:** None.

Poster

513. Synapse Formation: CNS II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.18/C28

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: CaMKII phosphorylation of neuroligin-1 regulates excitatory synapses

Authors: *M. A. BEMBEN[1], S. L. SHIPMAN[2], T. HIRAI[1], R. A. NICOLL[2], J. S. DIAMOND[1], K. W. ROCHE[1];
[1]NINDS, NIH, Bethesda, MD; [2]Cell. and Mol. Pharmacol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Neuroligins are postsynaptic cell adhesion molecules that are important for synaptic function through their transsynaptic interaction with neurexins. The localization and synaptic effects of neuroligin-1 are specific to excitatory synapses with the capacity to enhance excitatory synapses dependent on synaptic activity or Ca2+/CaM Kinase II. Here, we report that Ca2+/CaM

Kinase II robustly phosphorylates the intracellular domain of neuroligin-1 but not neuroligin-3. We show that T739 is the dominant CaMKII site and is phosphorylated in response to synaptic activity in neurons. Furthermore, a phospho-deficient mutant (T739A) reduces the basal and activity-driven surface expression of neuroligin-1, leading to a reduction in neuroligin-mediated excitatory synaptic potentiation. Therefore we report the first direct functional interplay between CaMKII and neuroligin-1, two primary components of excitatory synapses.

Disclosures: M.A. Bemben: None. **S.L. Shipman:** None. **T. Hirai:** None. **R.A. Nicoll:** None. **J.S. Diamond:** None. **K.W. Roche:** None.

Poster

513. **Synapse Formation: CNS II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 513.19/C29

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Intramural Funding

Title: Role of neurolastin, a novel brain-specific GTPase, in regulating excitatory synapses and spine morphology

Authors: *R. M. LOMASH, W. LU, R. J. YOULE, K. W. ROCHE;
NINDS, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Glutamate receptors mediate the majority of excitatory neurotransmission in the mammalian nervous system. The precise regulation of the levels of these receptors at synapses is the underlying mechanism mediating synaptic plasticity. Processes such as membrane trafficking and post-translational modifications including ubiquitination tightly regulate the number of neurotransmitter receptors available at any given time. Key players regulating membrane trafficking events include GTPases of the dynamin superfamily that hydrolyze GTP to steer membrane fusion events. Recently, a novel brain-specific GTPase was discovered in the Roche lab, which was named neurolastin (RNF112/Znf179) because it is most closely related to the dynamin superfamily GTPase, atlastin. Neurolastin also has a RING finger domain (typically found in E3 ligases, that mediate ubiquitination) and is the first identified protein with a unique domain organization harboring both GTPase and RING domains, yet there have been no reports in the literature that either annotate its GTPase domain or demonstrate its GTPase or E3 ligase activity. Here, we demonstrate that neurolastin has the ability to hydrolyze GTP to mono-phosphate (GMP) and the GTPase activity is involved in the maintenance of dendritic spine density. We observed that expression of neurolastin leads to an increase in the density of

dendritic spines on hippocampal neurons, whereas expression of the GTPase activity mutant (R340Q) did not affect the spine density demonstrating the role of neurolastin's GTPase activity in maintenance of spine density. Subsequently, we also observed a significant decrease in the frequency of mEPSCs in hippocampal slices from knockout mice indicating a marked reduction in the number of functional synapses in the absence of neurolastin. These results suggest that neurolastin affects synaptic plasticity by regulating synaptogenesis and spine maintenance. This work was initiated by Stephanie McNeil as part of her doctoral thesis at Johns Hopkins University.

Disclosures: R.M. Lomash: None. **W. Lu:** None. **R.J. Youle:** None. **K.W. Roche:** None.**Poster**

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.01/C30

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant AG039521 (B.A.J.)

NIH Grant MH081935 (P.E.C.)

Title: A novel role for AIDA-1 in regulating NMDA receptor subunit composition and long-term potentiation

Authors: *J. O. TINDI, A. E. CHÁVEZ, S. CVEJIC, P. E. CASTILLO, B. A. JORDAN; Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med. of Yeshiva Univ., Bronx, NY

Abstract: The NMDA subtype of ionotropic glutamate receptors (NMDARs) plays important roles in neurotransmission, synaptic plasticity, learning, memory and behavior. NMDARs are heterotetramers composed of two obligate GluN1 subunits together with GluN2 (A-D) and/or GluN3 (A-B) subunits, which confer specific channel properties. GluN1:GluN2A:GluN2B heteromers comprise the majority of NMDARs in the hippocampus, and the ratio of GluN2B to GluN2A subunits is developmentally regulated. How these subunits contribute to NMDAR function and higher order brain functions is unclear despite considerable efforts exploring this question. AIDA-1 (amyloid precursor protein intracellular domain associated -1) is widely expressed in the CNS, binds directly to the postsynaptic scaffolding protein PSD-95 and is present in complexes with NMDARs. Here we show that AIDA-1 is in complex with GluN2B but not GluN2A-containing NMDARs in the hippocampus. Acute knockdown of AIDA-1 in rat dissociated hippocampal neuronal cultures using lentivirally delivered shRNAs results in

decreased GluN2B subunit expression at synapses with a concomitant increase in GluN2A expression. We have also generated forebrain specific (using CaMKIIα-Cre mice) AIDA-1 conditional knockout mice (AIDA-1 cKO) to study the role of AIDA-1 *in vivo*. We find that Schaffer collateral to CA1 pyramidal neuron (Sch-CA1) synapses in these mice display normal basal AMPA receptor (AMPAR)-mediated transmission and no change in NMDAR/AMPAR current ratio. However, the NMDAR-EPSC decay time is reduced in AIDA-1 cKO mice, suggesting an alteration in the subunit composition of synaptic NMDARs. In addition, NMDAR-EPSCs in these mice shows reduced sensitivity to the GluN2B-specific antagonist Ro 25-6981, consistent with decreased GluN2B, increased GluN2A or both. Biochemical fractionation experiments reveal increased GluN2A and decreased GluN2B subunits in hippocampal postsynaptic densities from AIDA-1 cKO mice and no changes in GluN1 or other ionotropic glutamate receptors. The alteration in GluN2A/GluN2B expression ratio occurs without a change in total subunit abundance, suggesting a defect in NMDAR trafficking. Consistent with the critical role of NMDARs in synaptic plasticity, Sch-CA1 LTP is significantly impaired in AIDA-1 cKO mice. Moreover, AIDA-1 cKO mice exhibit deficits in memory-dependent tasks such as object placement recognition. Together, our findings support a novel synaptic role for AIDA-1 in the regulation of NMDAR composition at synapses with consequences for plasticity and behavior.

Disclosures: J.O. Tindi: None. **A.E. Chávez:** None. **S. Cvejic:** None. **P.E. Castillo:** None. **B.A. Jordan:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.02/C31

Topic: B.02. Ligand Gated Ion Channels

Support: NINDS intramural program

Title: A novel functional role of STEP in regulating phosphorylation of the synaptic GluN2B via PSD-95 association

Authors: *S. WON, K. W. ROCHE;
NIH/NINDS, NIH/NINDS, Bethesda, MD

Abstract: NMDA receptors (NMDARs) are a subtype of ionotropic glutamate receptors that play a key role in synaptic plasticity, neuronal development, learning and memory. They are functional tetramers composed of two GluN1 subunits, two GluN2A-D and GluN3 subunits.

Particularly, GluN2 subunits define many of the functional properties of the channel, their trafficking, and their synaptic expression. While the C terminus of GluN2B subunit of NMDARs contains a clathrin adaptor protein (AP-2) binding site and endocytic motif YEKL, GluN2A does not have the same motif. Many reports about tyrosine phosphorylation in the endocytic motif of GluN2B have revealed that this tyrosine phosphorylation affects surface expression of GluN2B-containing NMDARs. The tyrosine 1472 residue (Y1472) within the YEKL motif of GluN2B is phosphorylated by the Src family of kinases (SFKs). Particularly Fyn, a member of SFKs, can phosphorylate the Y1472 of GluN2B and the phosphorylation of Y1472 inhibits the binding of AP-2, thereby promoting surface expression of GluN2B-containing NMDARs. The phosphorylation of NMDARs by Fyn is promoted by postsynaptic density-95 (PSD-95), a member of membrane-associated guanylate kinase family of proteins (MAGUKs) that is well-known for binding to PDZ binding domain (ESDV) in the GluN2 subunits of NMDARs. Striatal-enriched protein tyrosine phosphatase (STEP) is a brain-specific intracellular tyrosine phosphatase. STEP has two representative isoforms, which are targeted to different intracellular compartments. $STEP_{46}$ is a cytosolic isoform, whereas $STEP_{61}$ is a membrane-associated isoform that localizes to the endoplasmic reticulum and the postsynaptic density. STEPs regulate ERK 1/2, p38, Fyn kinases and also GluN2B. STEP dephosphorylates ERK 1/2, p38, and Fyn kinases at their regulatory tyrosine residues, thereby inactivating them. In the case of GluN2B, STEP dephosphorylates Y1472 in its endocytic motif and facilitates the internalization of NMDARs including GluN2B subunits. However, the precise mechanism regulating the dephosphorylation of Y1472 in GluN2B by STEP is not understood. We have characterized the two isoforms and find a distinct subcellular localization in the rat brain and this implies they have different roles within the cell and at different developmental stages. We have also investigated the specificity of $STEP_{61}$ binding to a variety of MAGUKs and again find specificity of $STEP_{61}$ compared to $STEP_{46}$. The PDZ3 domain of PSD-95 binds to $STEP_{61}$ but not $STEP_{46}$. Interestingly, $STEP_{61}$ can also bind directly to the NMDARs. These results reveal the complexity of $STEP_{61}$ interactions with PSD-95 and NMDARs.

Disclosures: S. Won: None. **K.W. Roche:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.03/C32

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant R01 EY018441

NIH/NEI Grant 1-T32-EY14537

NIGMS Grant GM080202

Title: Specificity Protein 4 functionally regulates the transcription of NMDA receptor subunits GluN1, GluN2A, and GluN2B

Authors: *A. PRIYA, K. JOHAR, M. T. T. WONG-RILEY;
Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: N-methyl-d-aspartate (NMDA) receptors are major glutamatergic receptors involved in most excitatory neurotransmission in the brain. The transcriptional regulation of NMDA receptors is not fully understood. Previously, we found that the GluN1 and GluN2B subunits of the NMDA receptor are regulated by nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2). NRF-1 and NRF-2 also regulate all 13 subunits of cytochrome c oxidase (COX), a critical energy generating enzyme, thereby coupling neuronal activity and energy metabolism at the transcriptional level. Specificity Protein (Sp) is a family of transcription factors that bind to GC-rich regions, with Sp1, Sp3, and Sp4 all binding to the same cis motifs. Sp1 and Sp3 are ubiquitously expressed, whereas Sp4 expression is restricted to neuronal and testicular cells. Recently, we found that the Sp factors regulate all subunits of COX. The goal of the present study was to test our hypothesis that the Sp factors also regulate specific subunits of NMDA receptors, and that they function with NRF-1 and NRF-2 via one of three mechanisms: complementary, concurrent and parallel, or a combination of complementary and concurrent/parallel. By means of multiple approaches we found that Sp4 functionally regulates GluN1, GluN2A, and GluN2B, but not GluN2C. On the other hand, Sp1 and Sp3 did not regulate these subunits as previously thought. Our data indicate that Sp4 operates in a complementary as well as a concurrent and parallel manner with NRF-1 and NRF-2 to mediate the tight coupling between energy metabolism and neuronal activity at the molecular level. (Supported by NIH grant Grant R01 EY018441 and NIH/NEI Training Grant 1-T32-EY14537. Anusha Priya is a member of the MCW-MSTP which is partially supported by a T32 grant from NIGMS, GM080202.)

Disclosures: A. Priya: None. **K. Johar:** None. **M.T.T. Wong-Riley:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.04/C33

Topic: B.02. Ligand Gated Ion Channels

Support: Canadian Institutes of Health Research grant MT-12682 to MWS.

MWS holds a Canada Research Chair (Tier I).

MWS is the Anne & Max Tanenbaum Chair in Molecular Medicine.

Title: Identifying interacting regions between the GluN subunits & the Src anchoring protein ND2 in the NMDAR complex

Authors: *D. SCANLON, H. L. LEDUC-PESSAH, M. W. SALTER;
Neurosciences and Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Upregulation of NMDA receptors (NMDARs) by the tyrosine kinase Src is critical for long-term potentiation of synaptic transmission in the hippocampus & chronic pain hypersensitivity in the spinal cord. Src is anchored in the NMDAR complex by ND2, NADH dehydrogenase subunit 2, which functions as an adaptor protein. The primary sequence requirements for the interaction between Src & ND2 have been determined, but the interacting regions between ND2 & the GluN subunits have been elusive. To elucidate the basis for this interaction, we transfected HEK293 cells with GFP-tagged ND2 or GFP-ND2 fragments, with GluN subunits or receptor controls. We assessed the correlation between the ND2 constructs and the GluN subunits/controls by confocal microscopy. Thresholded Pearson's Correlation Coefficient (PCC) was used as a measure of colocalization. GFP-ND2 differentially colocalized with GluN1/GluN2A NMDARs (PCC = 0.61±0.03) as compared with AMPARs (0.19±0.03), P2X4Rs (0.30±0.03), actin (0.10±0.02) or PSD95 (0.28±0.03) (one-way ANOVA $p<0.001$). GFP-ND2 also differentially colocalized with GluN1 alone (0.66±0.02) as compared with GluN2A (0.03±0.03). Additionally, GFP-ND2 colocalized with a GluN1-C-terminal deletion mutant (0.65±0.03) and a GluN1 C and ATD (Amino Terminal Domain) deletion mutant (GluN1ΔCΔATD) (0.61±0.02). The ND2 fragment 150-347 (0.66±0.03) also colocalized with GluN1 alone, but the ND2 fragment 250-347 did not (0.33±0.04). Subsequently, we found that ND2-150-240 colocalized with GluN1 alone (0.78±0.02) and with both GluN1 + GluN2A (0.66±0.02) and GluN1ΔCΔATD + GluN2A (0.70±0.03). Thus, we have determined that GluN1, but not GluN2A, is necessary and sufficient for colocalization with ND2, and that the C-tail and amino terminal domain of GluN1 are dispensable. Furthermore, we have identified a 91 amino acid region of ND2 that is sufficient to interact with the core of the NMDAR complex.

Disclosures: D. Scanlon: None. **M.W. Salter:** None. **H.L. Leduc-Pessah:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.05/C34

Topic: B.02. Ligand Gated Ion Channels

Support: MIUR Projects # 2009R7WCZS_003

MIUR Projects #. 2009P7WHNR_003

Title: *In vitro* exposure to nicotine modulate the function of presynaptic NMDA receptors present on dopaminergic terminals in rat Nucleus Accumbens

Authors: *M. MARCHI[1], A. SALAMONE[1], S. ZAPPETTINI[1], M. GRILLI[1], G. OLIVERO[1], R. CUNHA[2], A. PITTALUGA[1];
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Abstract: Here we provide functional and immunocytochemical evidence supporting the presence on Nucleus Accumbens (NAc) dopaminergic terminals of N-methyl-D-aspartic acid (NMDA) receptors colocalized with nicotinic acetylcholine receptors (nAChRs). Immunocytochemical studies showed that a significant percentage of NAc terminals were dopaminergic and that most of these terminals also posses nAChRs which contain the α4 subunit. The in vitro short-term pre-exposure of synaptosomes to 30 µM nicotine or 5IA85380 caused a significant reduction of both the 30 µM nicotine and the 100 µM NMDA-evoked [^{3}H]Dopamine (DA) overflow. This reduction was completely counteracted when synaptosomes were pretreated with nicotine plus mecamylamine. In synaptosomes transiently stimulated with 100 µM NMDA before and after pretreatment with 100 µM nicotine or 10 nM 5IA85380 the time course of FURA-2 AM fluorescence emission changes shows a significant decrease of the NMDA-evoked calcium transients. The inhibitory effect of 5IA85380 was completely counteracted when synaptosomes were pretreated in the presence of the selective antagonist DHβE indicating that the changes of the NMDA-dependent DA release reported was dependent to the activation of a β2* nAChR subtype. The NMDA-evoked overflow was almost completely antagonized in presence of MK801 and partially inhibited in presence of the non specific antagonist CGS-19755 and by RO 25-6981 and Ifenprodil, two specific GluN2B antagonists. CPP-19755 and $ZnCl_2$ (1 nM), two compounds showing preferring affinities at GluN2A subunits did not antagonized the NMDA effect. A significant decrease in GluN2B biotin tagged proteins was observed following exposure of NAc synaptosomes to nicotine pretreatment when compared to control.. Therefore, our results show that the NMDA receptor function can be dynamically and negatively regulated in neurons in response to a brief incubation with nAChRs agonists.

Disclosures: M. Marchi: None. **A. Salamone:** None. **S. Zappettini:** None. **M. Grilli:** None. **G. Olivero:** None. **R. Cunha:** None. **A. Pittaluga:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.06/D1

Topic: B.02. Ligand Gated Ion Channels

Support: Repatriation CONACYT Grant 138425

Basic Science SEP-CONACYT Grant 132706

Title: N-Methyl-D-aspartate receptor is expressed by rat cultured cortical astrocytes and regulates their mitochondrial membrane potential

Authors: P. MONTES DE OCA BALDERAS[1], *P. AGUILERA[2], A. SANTAMARIA[1];
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Abstract: Astrocytes were conceived traditionally only as structural cells in the CNS. However, their gliotransmitter secretion, neurotransmitter receptor expression, and network properties provided the basis to support their role in information processing and cognitive functions. Ionotropic glutamate N-methy D-aspartate receptor (NMDAR) is critical in CNS functions including learning, memory and cognition. Nonetheless, NMDAR expression and functionality in astrocytes is still under research and debate. In this work, we investigated NMDAR subunit expression in rat cultured cortical astrocytes (rCCA) through immunofluorescence. Our observations demonstrate NMDAR subunits NR1, NR2A and NR2B labeling in GFAP positive cells with intracellular distribution characteristic of transmembrane molecules. Consistently, NMDAR subunit mRNA synthesis was found by qRT-PCR. Western Blot experiments with two different Abs flanking NR1 N-and C- terminal domains, the critical subunit for NMDAR assembly and transport, revealed its full length synthesis. Since these results indicated NMDAR expression in rCCA, we further assayed NMDAR functionality through time-lapse experiments evaluating astrocyte's mitochondrial membrane potential ($m\Delta\psi$) in response to NMDA using the ratiometric sensor JC-1. We found that NMDA treatment reduced $m\Delta\psi$, although cell-by-cell analysis revealed some response heterogeneity. NMDA effect on $m\Delta\psi$ was inhibited by NMDAR inhibitor MK-801 and accompanied by iCa++ rise measured with Fluo-4-AM. Altogether, these results demonstrate that rCCA express NMDAR subunits that are assembled into functional membrane receptors that after activation mediate extracellular Ca++ entry that diminishes $m\Delta\psi$. These observations open the possibility to use rCCA to study putative posttranslational modifications in NMDAR subunits.

Disclosures: P. Montes de Oca Balderas: None. **P. Aguilera:** None. **A. Santamaria:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.07/D2

Topic: B.02. Ligand Gated Ion Channels

Title: A novel partner for glun2a-containing nmda receptors, rnf10: a synapse-to-nucleus signal

Authors: F. GARDONI[1], M. C. DINAMARCA[1], F. GUZZETTI[1], D. LIM[2], J. STANIC[1], A. CALDARELLI[2], A. A. GENAZZANI[2], P. L. CANONICO[2], *M. DILUCA[1];
[1]Dept. of Pharmacologcial and Biomolecular Sci., Univ. of Milano, Milano, Italy; [2]Univ. degli Studi del Piemonte Orientale Amedeo Avogadro, Novara, Italy

Abstract: Among the cellular mechanisms required for modifications of dendritic spines, synapse-to-nucleus communication plays a key role in the regulation of the long-term structural changes. Emerging evidence indicates that multiple signalling pathways arising from dendritic spines converge to the nucleus regulating the expression of genes associated with changes of synapto-dendritic inputs. In the last decade, few synapto-nuclear protein messengers have been identified, and shown to play key roles in plasticity and synapse function. We recently identified Ring Finger Protein 10 (RNF10) as a new synapse-to-nucleus molecule, which responds to specific calcium signals at the postsynaptic compartment to elicit discrete changes at the transcriptional level. RNF10 is highly enriched at the excitatory synapse where it is associated to the GluN2A subunit of NMDA receptors. RNF10 is also present in the nucleus, where it is known to associate with Mesenchyme Homeobox 2 (Meox-2) transcription factor. Activation of synaptic NMDA receptors leads to RNF10 translocation from dendritic spines to the nucleus and induction of the expression of RNF10 target genes. Interestingly, modulation of RNF10 expression levels plays a fundamental role in regulating excitatory spine morphology under resting conditions as well as following activity-dependent synaptic plasticity.

Disclosures: F. Gardoni: None. **M. DiLuca:** None. **M.C. Dinamarca:** None. **F. Guzzetti:** None. **J. Stanic:** None. **D. Lim:** None. **A. Caldarelli:** None. **A.A. Genazzani:** None. **P.L. Canonico:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.08/D3

Topic: B.02. Ligand Gated Ion Channels

Support: T32GM007108

Title: Mechanisms of synaptic NMDA receptor potentiation by Wnt5a

Authors: A. MCQUATE[1], *A. BARRIA[2];
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Abstract: Wnts are secreted glycoproteins that signal through canonical and noncanonical pathways to organize synapses in the adult nervous system. Wnt5a specifically and acutely potentiates NMDA receptor transmission at the hippocampal CA3-CA1 synapse. The potentiation requires intracellular calcium, PKC, and JNK kinases. The precise mechanisms leading to larger NMDAR current amplitudes, however, remain unknown. We hypothesized that Wnt5a facilitates trafficking of NMDA receptors into the synapse. We found the increase in NMDAR currents after Wnt5a application is dependent on SNAP-25, a member of the SNARE family of membrane fusion proteins. Using two-photon fluorescence microscopy and optically tagged NR2 subunits of the NMDA receptor, we are further testing whether Wnt5a induces surface expression or surface redistribution of NMDA receptors. Overall, our results support the hypothesis that Wnt5a can regulate glutamatergic synapses in the adult nervous system by governing NMDA receptor trafficking. This novel role for Wnt signaling could have profound consequences in regulating both basal synaptic transmission and plasticity.

Disclosures: A. McQuate: None. **A. Barria:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.09/D4

Topic: B.02. Ligand Gated Ion Channels

Title: Effects of polyamines and monoamines on GluN1/GluN2A and GluN1/GluN2B subtypes of NMDA receptor

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Abstract: NMDA-type glutamate receptors (NMDA receptors) are widely distributed in the central nervous system and play critical roles in synaptic plasticity and excitotoxicity. Dysfunctions of NMDA receptors are involved in several central nervous system disorders, including stroke, chronic pain and schizophrenia. There are a few NMDA receptor antagonists available for clinical use, including ketamine and most importantly, mematine, both of which act as channel blockers. Mematine is used for Alzheimer's disease.Polyamines, such as putrescine, spermine and spermisine, are poly basic aliphatic amines that were ubiquitously present in prokaryotic and eukaryotic cells. Extracellular polyamines have multiple effects on NMDA receptors. Spermine is known to inhibit or activate the channel activity of NMDA receptor subunits dependent manner.
Only NMDA receptors containing the GluN2B subunits display polyamine potentiation. The study of the channel block activity with the polyamine derivative is applied to a study of the structure activity correlation of the receptor, development of the brain function or improvement medicine. Wine, sake, cheese, and other fermented foods are known to contain various polyamines and monoamines. Junmai shu (Japanese sake made from rice only) contains several amines (agmatine, spermine, putrescine, tyramine, isoamylamine, and 2-phenylethylamine). Some of them have not investigated about their physiological activity. So, we examined the effect of six amines on GluN1/GluN2B and GluN1/GluN2A subtypes of NMDA receptors, which were expressed in *Xenopus* oocytes. Agmatine, putrescine, tyramine, and 2-phenylethylamine inhibited the activity of both receptors. 2-phenylethylamine especially inhibited the activity of both receptors.
Isoamylamine indicated the opposite effect of spermine. It was indicated that isoamylamine act as an antagonist against the GluN1/GluN2B receptor, but an activator for GluN1/GluN2A receptor. Observed differences in activity between the receptor subtypes, it may be able to elucidate the mechanism of action of amines on the NMDA receptor channel. It was indicated the possibility that activator amines used as medicines for mental illness, such as schizophrenia and antagonistic amines used as potential therapeutic agents such as anxiety and dementia.

Disclosures: Y. Yamada: A. Employment/Salary (full or part-time):; full. **K. Norikane:** None. **K. Matsumaru:** None. **H. Izu:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.10/D5

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant NS032123

Attilio and Olympia Ricciardi Fund

Title: NMDAR-mediated EPSCs in single pyramidal neurons are highly dependent on a concomitant rise in peri-synaptic pH

Authors: H.-Y. CHEN[1], *M. CHESLER[2];
[1]Dept. of Physiol. & Neurosci., NYU Sch. of Med., New York, NY; [2]Dept. of Neurosurg., Dept. Neurosurg. NYU Sch. of Med., New York, NY

Abstract: Synchronous neural activity elicits a population extracellular alkaline transient (PAT) due to proton – Ca^{2+} exchange of the plasma membrane Ca^{2+} ATPase (PMCA). Previous work on hippocampal CA1 pyramidal neurons (PNs) indicated that a PAT boosts the NMDAR-mediated component of EPSCs elicited via the Schaffer collaterals, presumably due to relief of the constitutive proton block of these receptors (1). There was no such effect on the AMPAR component of the current (2,3). Whether this modulation of post-synaptic NMDARs occurs with more discrete activity is unknown. To test this idea we blocked the PAT with DNQX plus picrotoxin (to shut down all population activity) and evoked pure NMDAR-mediated EPSCs in single PNs clamped at +50 mV. Increasing effective buffering capacity with exogenous carbonic anhydrase (xCAR) decreased the EPSC peak by 32 $\pm$ 3.3 percent (N=8 cells) with no change in time course. xCAR caused similar reduction of EPSCs at holding potentials of +50 versus –30 mV, and did not alter EPSC reversal potential. The effect of adding xCAR was: (i) blocked by benzolamide, an inhibitor of extracellular carbonic anhydrase, (ii) mimicked by 20 mM HEPES in standard Ringer, and (iii) was fully occluded by HEPES in standard Ringer. These data indicate that the effect of xCAR was due to pH buffering, and required the enzyme active site. Given that buffering can reduce, but not abolish a peri-synaptic pH change, our results indicate that post-synaptic NMDAR current of a single CA1 PN is highly dependent on a self-generated rise in peri-synaptic pH. These data further suggest that the PMCA, due to its inward transport of protons, can paradoxically boost Ca^{2+} entry via NMDARs, despite its more general, evolved role as a mechanism of cytosolic Ca^{2+} clearance. (1) Traynelis et al. (2010). Pharmacol. Reviews 62, 405-496. (2) Makani and Chesler (2007). J. Neurosci. 27(28):7438-7446 (3) Makani and Chesler (2010). J Neurophysiol 103: 667–676.

Disclosures: H. Chen: None. **M. Chesler:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.11/D6

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant NS065371

NIH Grant DA015040

NIH Grant GM008602

Lundbeck

the Brain and Behavior Research Foundation (NARSAD)

Title: Subunit-selective glun2c and glun2d nmda receptor potentiators reverse mk801-induced impairment of pre-pulse inhibition

Authors: *K. STRONG[1], K. K. OGDEN[2], R. M. SANTANGELO FREEL[1], D. LIOTTA[1], L. G. CHASTAIN[3], B. KINKEAD[3], S. F. TRAYNELIS[2];
[1]Chem. Dept., [2]Pharmacol. Dept., [3]Psychiatry and Behavioral Sci., Emory Univ., Atlanta, GA

Abstract: NMDA receptors mediate a slow, Ca^{2+}-permeable component of excitatory synaptic transmission. One leading hypothesis concerning the basis of schizophrenia postulates that NMDA receptor hypofunction underlies certain clinical features of the disease. This hypothesis has led to the prediction that NMDA receptor potentiators could have therapeutic benefit for treatment of schizophrenia. We recently reported a new class of GluN2C/D-selective potentiators built on a tetrahydroisoquinoline core. Here we describe in detail the structure-activity relationship around this series. We also report stereoselective actions of several members of this class of potentiators. We further show that these potentiators are more effective at low agonist concentrations. Analysis of off-target activity and brain penetration (brain:plasma ratio of >2) supports the use of these compounds in animal models that are relevant for schizophrenia. We therefore evaluated whether a prototypical member of this class (CIQ) could rescue deficits of pre-pulse inhibition (PPI), a measure of sensorimotor gating, caused by the use-dependent NMDA channel blocker MK801. Compared to controls (19.5% PPI), we find robust disruption of PPI by 0.2 mg/kg (sc) MK801 (4.1% PPI) that is significantly relieved by administration of 20 mg/kg CIQ i.p (14.8% PPI, n=14-15, $p<0.05$ ANOVA). These data support the idea that NMDA receptor potentiation might have utility in treatment of schizophrenia, and further highlight GluN2C/D subunits as important contributors to the effects of MK801 on PPI

Disclosures: K. Strong: None. **K.K. Ogden:** None. **R.M. Santangelo Freel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

excluding diversified mutual funds); co-inventors on Emory owned IP (WO2010/088414 A2). **D. Liotta:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp Inc., Atlanta GA, co-inventors on Emory owned IP (WO2010/088414 A2). F. Consulting Fees (e.g., advisory boards); NeurOp Inc, Atlanta GA. **L.G. Chastain:** None. **B. Kinkead:** None. **S.F. Traynelis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp Inc., Atlanta GA, co-inventors on Emory owned IP (WO2010/088414 A2). F. Consulting Fees (e.g., advisory boards); NeurOp Inc, Atlanta GA.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.12/D7

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant DA726121

NIH Grant MH78823

NIH Grant GM47969

Bantly Foundation

Sage Therapeutics

Title: Mechanisms of oxysterol modulation of NMDA receptor function

Authors: *A. J. LINSENBARDT[1], H.-J. SHU[1], J. J. DOHERTY[3], S. M. PAUL[3,4], C. F. ZORUMSKI[1,2,5], S. MENNERICK[1,2,5];
[1]Dept. of Psychiatry, [2]Dept. of Anat. & Neurobio., Washington Univ., Saint Louis, MO; [3]Sage Therapeut., Cambridge, MA; [4]Appel Alzheimer's Dis. Res. Inst., Weill Cornell Med. Col., New York, NY; [5]Taylor Family Inst. for Innovative Psychiatric Res., Saint Louis, MO

Abstract: N-methyl-D-aspartate receptors (NMDARs) are heterotetrameric ligand-gated ion channels implicated in learning and memory, as well as in a variety of neuropsychiatric disorders including schizophrenia and Alzheimer’s disease. NMDARs are gated by the neurotransmitter glutamate and are positively modulated by endogenous hydrophobic allosteric ligands including pregnenolone sulfate (PS) and arachidonic acid (AA). Recently, we have discovered a novel endogenous NMDAR positive allosteric modulator, the major brain cholesterol metabolite 24(S)-hydroxycholesterol (24(S)-HC). We have also shown that the synthetic oxysterol derivative [$\Delta^{5,6}$-

3β-Oxy-nor-cholenyl]-dimethyl-carbinol (SGE-201) similarly modulates NMDAR function. Here we investigate additional mechanistic aspects of oxysterol regulation of NMDARs. These oxysterols exhibited no selectivity for the major NMDAR GluN2 subunits (GluN2A, B, C, D), suggesting a broad spectrum of action. Potentiation was observed in subsaturating and saturating concentrations of glycine, suggesting that oxysterol potentiation does not involve interaction with co-agonist. Oxysterol modulation also did not depend on NMDA concentration, suggesting that potentiation is associated with an increase in agonist efficacy. In tests of shared mechanism we found that SGE-201 and 24(S)-HC exhibit occlusion, yet oxysterols did not occlude potentiation by PS or by the membrane-derived phospholipid, AA. Another endogenous oxysterol, 25-hydroxycholesterol did not potentiate NMDAR activity but occluded modulation by SGE-201. This suggests possible competition among different endogenous cholesterol metabolites and excludes shared actions of oxysterols (e.g., membrane disordering effects) in NMDAR potentiation. Although intracellular access could participate in oxysterol actions, SGE-201 potentiation required extracellular access, suggesting an extracellular binding site. Intracellular application of SGE-201 to neurons was ineffective. In excised outside-out patches, SGE-201 potentiated channel activity without significantly altering channel conductance, also consistent with a direct action on NMDAR channel gating. Our data demonstrate that 24(S)-HC and the structurally-related oxysterol SGE-201 act through a previously undescribed mechanism and are unusual among positive allosteric modulators of ligand-gated ion channels in regulating agonist efficacy without appreciable effects on channel closing rate.

Disclosures: A.J. Linsenbardt: None. **H. Shu:** None. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **S.M. Paul:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **C.F. Zorumski:** F. Consulting Fees (e.g., advisory boards); Sage Therapeutics. **S. Mennerick:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Sage Therapeutics.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.13/D8

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant GM008151

NIH Grant MH078823

NIH Grant MH077791

NIH Grant AA01743

The Bantly Foundation

Title: Network pharmacodynamics of the NMDAR channel blockers memantine and ketamine

Authors: *C. M. WROGE[1,2], L. N. EISENMAN[3], Y. IZUMI[1,5], J. J. DOHERTY[6], S. M. PAUL[7], C. F. ZORUMSKI[1,5], S. MENNERICK[1,4,5];
[1]Psychiatry, [2]Grad. Program in Neurosci., [3]Neurol., [4]Anat. and Neurobio., Washington Univ., Saint Louis, MO; [5]Taylor Family Inst. for Innovative Psychiatric Res., Saint Louis, MO; [6]Sage Therapeut., Cambridge, MA; [7]Appel Alzheimer's Dis. Res. Inst., New York, NY

Abstract: Memantine and ketamine, activation dependent blockers of the NMDA receptor, have markedly different properties clinically. Mild pharmacodynamic differences at steady-state have been suggested to participate in clinical differences, but it is unknown whether these differences are evident during non-steady state agonist presentation and transient depolarization that characterize physiological activity. We previously found no difference between memantine and ketamine, except that memantine exhibits slightly faster re-equilibration following depolarization. Consistent with the suggestion that this biophysical difference plays little role under physiological conditions, the drugs were indistinguishable when network activity was measured as spontaneous EPSCs or using a multi-electrode array. At low micromolar concentrations and with acute application 15 prior to induction, both drugs also selectively inhibited LTD but not LTP in hippocampal slices, again denoting essential similarity under dynamic conditions. We were only able to discern a slight but statistically significant difference between the drugs in their neuroprotection against hypoxic insult. We hypothesize that these drugs do not escape the channel during EPSPs in part because of the weak gating efficacy of the NMDAR channel. We manipulated channel gating efficacy by chelating Zn2+ at GluN2A-containing receptors or with the synthetic oxysterol analogue: δ5,6-3β-Oxy-nor-cholenyl]-dimethyl-carbinol (SGE-201). Both treatments increased the rate constant of drug re-equilibration upon depolarization. For instance, SGE-201 decreased memantine (10 μM) steady-state block at -70 mV (96 ± 0.7% to 92 ± 1%, $p<0.05$) and reduced the weighted time constant for drug re-equilibration at +50 mV from 1293 ± 250 ms to 384 ± 45 ms ($p<0.05$, N=11). SGE-201 also enhanced separation between the neuroprotective effects of ketamine and memantine, consistent with our biophysical observations. We conclude that differences in voltage dependence between the two drugs are masked by the inherently low gating efficacy of the NMDA receptor, which is unusual among ligand-gated channels. Under baseline conditions the drugs act essentially voltage independently over the time course of EPSPs.

Disclosures: C.M. Wroge: None. **L.N. Eisenman:** None. **Y. Izumi:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Sage Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **S.M. Paul:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **C.F. Zorumski:** F. Consulting Fees (e.g., advisory boards); Sage Therapeutics. **S. Mennerick:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Sage Therapeutics.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.14/D9

Topic: B.02. Ligand Gated Ion Channels

Support: Sage Therapeutics

NIH Grant MH078823

NIH Grant MH077791

NIH Grant GM47969

NIH Grant AA017413

Bantly Foundation

Title: The major brain cholesterol metabolite 24(S)-hydroxycholesterol is a potent allosteric modulator of N-methyl-D-aspartate receptors

Authors: *S. M. PAUL[1,2], J. J. DOHERTY[2], A. J. ROBICHAUD[2], G. BELFORT[2], B. Y. CHOW[2], D. C. CRAWFORD[3], A. J. LINSENBARDT[3], Y. IZUMI[3], S. MENNERICK[3], C. F. ZORUMSKI[3];
[1]Appel Alzheimer's Dis. Res. Inst., New York, NY; [2]Sage Therapeut., Cambridge, MA; [3]Washington Univ. Sch. of Med., St. Louis, MO

Abstract: N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that are critical to the regulation of excitatory synaptic function in the CNS. NMDARs are essential for

experience-dependent synaptic plasticity and have also been implicated in the pathophysiology of various neuropsychiatric disorders including the cognitive deficits of schizophrenia and Alzheimer's disease as well as certain forms of autism. NMDARs are modulated by a number of endogenous substances including Mg^{2+} and the co-agonists glycine and D-serine. Certain neurosteroids have been shown to modulate NMDARs experimentally, both *in vitro* and *in vivo*, but their low potency and very low brain concentrations make them poor candidates as endogenous ligands. We now report that the major brain-derived oxysterol 24(S)-hydroxycholesterol (24(S)-HC) is a very potent and selective positive allosteric modulator of NMDARs. At nanomolar to low micromolar concentrations, well below that present in brain, 24(S)-HC potentiates with unusually slow kinetics NMDAR-mediated EPSCs in mouse hippocampal neurons but fails to affect AMPAR or GABA-AR-mediated responses. Cholesterol itself and other naturally-occurring oxysterols present in brain do not modulate NMDARs at concentrations ≤ 10 μM. Modulation appears to involve a direct effect on NMDARs rather than second-messenger signaling or transcription, as NMDAR potentiation is evident in excised outside-out membrane patches. In hippocampal slices, 24(S)-HC enhances the ability of subthreshold stimuli to induce long-term potentiation (LTP), a form of synaptic plasticity underlying learning and memory. 24(S)-HC also prevents a deficit in LTP induction triggered by transient application of low micromolar ketamine that persists for several hours following ketamine washout. Finally, we show that two synthetic drug-like derivatives of 24(S)-HC (SGE-201 and SGE-301) which potently enhance NMDAR-mediated-EPSCs and LTP via an overlapping if not identical oxysterol modulatory site, reverse memory impairment induced by a NMDAR antagonist in mice. 24(S)-HC is synthesized from cholesterol by cholesterol 24-hydroxylase a cytochrome P450 enzyme (CYP46A1) expressed primarily in brain and localized to the endoplasmic reticulum of neuronal cell bodies and dendrites. Consequently, 24(S)-HC may function as an endogenous autocrine modulator of NMDARs acting at a novel oxysterol modulatory site that also represents a potential target for therapeutic drug development.

Disclosures: **S.M. Paul:** A. Employment/Salary (full or part-time):; Weill Cornell Medical College. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **J.J. Doherty:** None. **A.J. Robichaud:** None. **G. Belfort:** None. **B.Y. Chow:** None. **D.C. Crawford:** None. **A.J. Linsenbardt:** None. **Y. Izumi:** None. **S. Mennerick:** None. **C.F. Zorumski:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.15/D10

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant NS036654

NIH Grant NS065371

NIH Grant GM008602

Michael J. Fox Foundation

Pfizer Inc.

Lundbeck AS

Title: A novel class of positive allosteric NMDA receptor modulators

Authors: *R. E. PERSZYK[1], B. M. KATZMAN[2], D. C. LIOTTA[2], S. F. TRAYNELIS[1]; [1]Pharmacol., [2]Chem., Emory Univ., Atlanta, GA

Abstract: N-methyl-D-aspartate (NMDA) receptors are ionotropic ligand-gated ion channels that are activated by glutamate and glycine and mediate a slow component of excitatory synaptic currents in the central nervous system. These receptors play important roles in synaptic plasticity, neuronal development, and have been implicated in a number of neurological disorders. The enhancement of NMDA receptor-mediated currents has been hypothesized to confer cognitive enhancement and could be potentially beneficial in correcting discrepancies in schizophrenia. Testing these hypotheses has been limited due to a lack of highly potent and effective pharmacological compounds. We have identified a number of structurally distinct classes of NMDA receptor allosteric modulators through a screen of 100,000 compounds. During the structure-activity relationship development of one of these classes of negative allosteric modulators (typified by compound 1794-1) we discovered a series of substitutions on the molecule that converted these negative allosteric modulators into positive allosteric modulators. Using two-electrode voltage clamp recordings of NMDA receptors expressed in *Xenopus oocytes*, we have studied several of these related NMDA receptor potentiators and found that they can enhance responses 150-250% with EC_{50} values ranging from 2 to 10 μM (n=10-14). Co-application of positive and negative modulators in this class suggests that the positive modulators can compete with the negative modulators. This raises the possibility that both positive and negative modulation of NMDA receptors by this class of molecules occurs through actions at a single site. This class of compounds could be utilized to assess the validity of enhancing NMDA receptor activity as a potentially beneficial strategy in neuropsychiatric disorders, and could lead to the development of drug-like pharmacological compounds targeting these strategies.

Disclosures: R.E. Perszyk: None. **B.M. Katzman:** None. **D.C. Liotta:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp Inc. F. Consulting Fees (e.g., advisory boards); NeurOp Inc.

S.F. Traynelis: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp Inc. F. Consulting Fees (e.g., advisory boards); NeurOp Inc.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.16/D11

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant GM008602

NIH Grant DA015040

NIH Grant NS036654

Title: The pre-M1 region of GluN2 subunits is a critical gating element of NMDA receptors

Authors: *K. K. OGDEN, J. ZHANG, S. F. TRAYNELIS;
Pharmacol., Emory Univ., ATLANTA, GA

Abstract: Glutamate receptor ion channels, which include AMPA receptors, kainate receptors, and NMDA receptors, mediate the majority of excitatory synaptic transmission in the central nervous system. A fundamental question is how these ligand-gated ion channels transduce binding of the amino acid glutamate into opening of the ion channel pore. X-ray crystallographic data from a tetrameric AMPA receptor revealed a short 7 residue "cuff" helix in the pre-M1 region of the receptor that is situated parallel to the lipid bilayer and may act a critical structural determinant of channel gating by restricting movement of pore-forming transmembrane helices until agonist binds. Whether a similar element exists in NMDA receptors and what its role in gating is remains unknown. Using cell-attached patch clamp, we recorded single-channel currents from NMDA receptors containing mutations in the pre-M1 region of GluN2A. Mutations at several residues in the pre-M1 region to alanine or to the homologous residue in the GluN2D subunit reduced channel open probability. The most dramatic changes occurred with 2A(L550A), which reduced open probability from 0.12 ± 0.05 (control, n=7) to 0.0012 ± 0.0003 (n=7). Open durations were described by a mixture of two exponential distributions with $\tau 1$=1.54 ms (area=74%) and $\tau 2$=0.12 ms for control. The fitted time constants were markedly shorter in 2A(L550A), with $\tau 1$=0.55 ms (area=66%) and $\tau 2$=0.10 ms. These data suggest the pre-M1 region of GluN2A is critical for normal gating of NMDA receptors. The pre-M1 region of GluN2D was recently implicated in the actions of the GluN2C/2D-selective positive allosteric

modulator CIQ. Thus, understanding how the pre-M1 region impacts gating of NMDA receptors may reveal new mechanisms for regulation of the receptor by endogenous and exogenous modulators and interacting proteins.

Disclosures: K.K. Ogden: None. **J. Zhang:** None. **S.F. Traynelis:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.17/D12

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant NS036654

NIH Grant NS065371

NIH Grant GM008602

Michael J. Fox Foundation

Pfizer, Inc.

Lundbeck AS

Title: A novel class of negative allosteric modulators of NMDA receptor function

Authors: *B. M. KATZMAN[1], R. E. PERSZYK[2], D. C. LIOTTA[1], S. F. TRAYNELIS[2]; [1]Chem., [2]Pharmacol., Emory Univ., Atlanta, GA

Abstract: The N-methyl-D-aspartate receptor (NMDA receptor) is an ionotropic glutamate receptor that mediates a slow component of excitatory synaptic transmission. Under normal situations, NMDA receptors are involved in many neurological processes including learning and memory. NMDA receptor dysfunction has been implicated in several neuropathological conditions, such as Parkinson's disease, Alzheimer's disease, and neuronal damage during ischemia. Therapeutic agents acting at NMDA receptors have been proposed for treating disorders, but in most cases side effects have complicated the clinical evaluation. In an effort to identify new modulators of NMDA receptors, we screened a library of compounds against GluN1/GluN2C- and GluN1/GluN2D-expressing BHK cells. Identification of two screening hits (compound 1794-1 and 1794-2), lead to the development of a novel class of inhibitors that have a modest preference for GluN2C/D receptors. These compounds are noncompetitive inhibitors of

NMDA receptor function, as their actions are not surmounted by increasing the concentration of glutamate or glycine (n=4-6 for all). Additionally, evaluation of the current-voltage curve showed that inhibition was voltage-independent for 1794-2 (3 μM, n=5), suggesting these compounds do not block the open channel. Interestingly, multiple members of this class of compounds cause sub-maximal inhibition even at saturating concentrations in two-electrode voltage clamp recordings of *Xenopus oocytes* expressing recombinant receptors. We have shown one member of this series (1794-2) is active in neurons, inhibiting NMDA receptor function in cerebellar granule cells with an IC_{50} value (3.5 μM, n=2-5) similar to that at recombinant GluN2A- and GluN2B-containing recombinant receptors (1 μM n=18, 3 μM n=12, respectively). Compound 1794-2 also shows neuroprotective properties against NMDA-induced toxicity in primary neuronal cultures (n=2). This class of compounds could be useful neuroprotective agents in stroke or neurodegenerative diseases, with their partial antagonism potentially diminishing undesirable side effects.

Disclosures: B.M. Katzman: None. **R.E. Perszyk:** None. **D.C. Liotta:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp, Inc.. F. Consulting Fees (e.g., advisory boards); NeurOp, Inc. **S.F. Traynelis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp, Inc.. F. Consulting Fees (e.g., advisory boards); NeurOp, Inc., Sage Therapeutics.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.18/D13

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant RO1NS052669

Title: Modulation of glycinergic NMDA receptors by protons and zinc

Authors: *K. A. CUMMINGS, G. K. POPESCU;
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Abstract: Glycinergic N-methyl-D-aspartate receptors (NMDARs) differ from the traditional glutamatergic NMDARs in that that they are insensitive to glutamate and can be activated by glycine alone. They assemble as combinations of eight GluN1 (NR1) splice variants (1a through 4b) and two GluN3 (NR3) isoforms (A and B). NR1/NR3 receptors have smaller unitary conductance, lower Ca2+ permeability, and decreased sensitivity to voltage-dependent Mg2+

block. Zinc and hydrogen ions are endogenous modulators of synaptic transmission and are strong allosteric inhibitors of classical NMDARs. Here we investigated the effects of zinc and hydrogen ions on GluN1-4a/GluN3A (NR1/NR3A) receptors. We found that in contrast to classical NMDARs, protons potentiated glycine-elicited NR1/NR3A receptor currents ~ 2-fold (Imax ~ pH 6.8), and also decreased the rate and extent of their desensitization. At physiological proton concentrations (pH 7.4), zinc ions (50 uM) inhibited the glycine-elicited current by ~60% and this effect was absent at pH 6.8. The zinc effects we observed differ from previously reported strong potentiation by zinc in glycinergic receptors assembled from the NR1-1a splice variant. Because the 1a and 4a splice variants only differ in the intracellular C-terminal tail, taken together with known spatiotemporal expression of the NR1 splice variants, these results suggest a possible intracellularly-governed response to extracellular zinc that may be brain region-specific. Additionally, these results indicate that under both physiological and pathological conditions, NR1/NR3A receptors function on a broad spectrum of activity. Increased activity of these receptors under periods of low pH such as stroke indicates that these receptors may play a larger role in ischemic events than previously known, making NR1/NR3A receptors a viable drug target.

Disclosures: K.A. Cummings: None. **G.K. Popescu:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.19/D14

Topic: B.02. Ligand Gated Ion Channels

Support: Wellcome Trust

Medical Research Council

Biotechnology and Biological Sciences Research Council

Title: Development of NMDA receptor-dependent glutamate excitotoxicity in human embryonic stem cell-derived neurons: An *In vitro* model system

Authors: *K. GUPTA[1,2], G. E. HARDINGHAM[3], S. CHANDRAN[2];
[1]MRC Lab. for Regenerative Med., Univ. of Cambridge, Cambridge, United Kingdom; [2]Ctr. for Neuroregeneration, [3]Ctr. for Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Increasing evidence implicates the role of glutamate-mediated excitotoxic neuronal death in the pathogenesis of a range of neurological disorders. Neuronal excitotoxicity is principally mediated by the NMDA-subtype glutamate receptor (NMDAR), though other pathways have been implicated. Evidence suggests that the subunit composition of NMDARs is critical for downstream signaling, and as such physiological patterns of synaptic glutamate transmission can be neuroprotective, while aberrant glutamate transmission can result in neuronal death. Human based experimental systems, specifically human pluripotent stem cells, have the potential to further elucidate pathways relevant to human neurological diseases and identify novel therapeutic targets. In this study, we utilize human embryonic stem cells (HESC) to generate an enriched population of neurons and characterize the development of functional glutamate responses and vulnerability to glutamate excitotoxicity. We demonstrate that HESC-derived neurons progressively developed functional glutamate responses attributable to NMDAR activity over two months in culture, concomitant with increasing expression of NMDAR and AMPAR receptor subunit mRNA. Moreover, differentiated human neurons exhibited dose-dependent glutamate-mediated cell death within the range of glutamate concentrations found in human patients suffering acute brain injury. NMDAR antagonists varied in efficacy; MK801 significantly reduced excitotoxic neuronal death. The NR2B-selective antagonist ifenprodil did not significantly reduce neuronal excitotoxic cell death. Interestingly, NMDA alone did not recapitulate the full excitotoxic effect of glutamate in this system. These data highlight the complexity of NMDAR and excitotoxic signaling, and demonstrate the utility of a human in vitro platform for enhancing the clinical translation of findings made in complementary systems. Furthermore, this study provides important proof-of-concept that HESC-derived neurons can be used effectively to model glutamate-mediated neurotoxicity and test candidate therapeutic compounds, and can potentially inform future studies in other human pluripotent systems such as induced pluripotent stem cells.

Disclosures: K. Gupta: None. **G.E. Hardingham:** None. **S. Chandran:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.20/D15

Topic: B.02. Ligand Gated Ion Channels

Support: NIH NS 26494

NIH MH 46613

Title: Kinetics and pharmacology of triheteromeric nmda receptors at hippocampal synapses

Authors: *K. R. TOVAR[1], G. L. WESTBROOK[2];
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Abstract: The majority of NMDA receptor activation occurs at synapses. The transient nature of quantal neurotransmitter presentation and elimination imposes specific macroscopic behavior on the resulting excitatory postsynaptic current (EPSC), which is governed by the subunit composition of synaptic receptors. NMDA receptors are tetrameric proteins composed of GluN1 and GluN2 subunits, in a 1 to 1 ratio. NMDA receptor functional heterogeneity results from developmental and anatomical expression patterns of GluN2 subunits. Additionally, many neurons in the mammalian central nervous system express more than one GluN2 subunit, leading to the possibility of three NMDA receptor subtypes at synapses. In mouse hippocampal neurons expressing GluN2A and GluN2B, most synaptic NMDA receptors contain both GluN2 subunits, along with GluN1 (Tovar et al., 2013). In isolation, these receptors have kinetic and pharmacological properties that are distinct from diheteromeric receptors containing GluN2A or GluN2B. GluN2A and GluN2B are the most highly expressed GluN2 subunits in the mammalian brain and thus synaptic triheteromeric receptors with distinct properties may be more prevalent than previously appreciated. The amino-terminal domains (NTDs) of GluN2 subunits, in large measure, defines subunit-dependent properties. NTDs are the binding sites for ligands such as zinc or ifenprodil which modulate NMDA receptor gating behavior. Ligand interaction with these domains prolong the activation of NMDA receptor-mediated EPSCs in diheteromeric receptors. This results from an increase in the channel dwell time in agonist-bound, nonconducting states (Tovar and Westbrook, 2012). In wild-type neurons, zinc or ifenprodil reduced the EPSC amplitude of isolated triheteromeric receptors containing GluN2A and GluN2B (AB-type receptors). However zinc prolonged EPSCs whereas ifenprodil did not. Thus the effect of ifenprodil on AB-type EPSCs compares to that of a competitive antagonist rather than a gating modulator. The dichotomy of effectiveness between NTD ligands is interesting in terms of screening therapeutics that modulate NMDA receptor kinetic behavior. To extend these observations, we are investigating whether other phenylethanolamine compounds cause prolonged activation of NMDA receptor-mediated EPSCs in isolated triheteromeric receptors, as well as how they affect gating in the non-equilibrium environment. This work could address why phenylethanolamine compounds have thus far, not proven successful as clinical therapeutics and point toward a more effective drug discovery strategy.

Disclosures: K.R. Tovar: None. **G.L. Westbrook:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.21/D16

Topic: B.02. Ligand Gated Ion Channels

Support: New Jersey Commission on Brain Injury Research

Autism Speaks

Alfred P. Sloan Foundation

Title: MHC class I is a voltage-dependent regulator of NMDA receptor-mediated single-channel currents

Authors: *C. M. TYLER[1], M. CHACON[1], J. I. AGUILAR[1], D. H. PERLMAN[1], L. FOURGEAUD[2], L. M. BOULANGER[1];
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Abstract: Proteins of the major histocompatibility complex class I (MHC class I) are known for their role in the immune response, and were recently identified as endogenous regulators of excitatory synaptic transmission. In hippocampal CA1 pyramidal neurons from MHC class I-deficient ($\beta2M^{-/-}TAP^{-/-}$) mice, whole-cell currents mediated by NMDA-type glutamate receptors (I_{NMDAR}) are significantly enhanced, while currents mediated by AMPA-type glutamate receptors are unaffected. The increase in whole-cell I_{NMDAR} in MHC class I-deficient brain is not associated with changes in the levels, localization, or subunit composition of NMDARs, or in the number of AMPAR-free ("silent") synapses (Fourgeaud *et al.*, 2010). Thus the cellular mechanisms by which MHC class I regulates whole-cell I_{NMDAR} remain unknown. One key question is whether MHCI affects NMDAR-mediated currents equally across a range of postsynaptic membrane potentials. We find that whole-cell I_{NMDAR} is selectively enhanced at depolarized membrane potentials (+40 mV), but not at hyperpolarized potentials (-70mV), in MHC class I-deficient mice, consistent with the idea that MHC class I does not affect NMDAR expression or trafficking, but rather might influence NMDAR single-channel behavior. To test if MHC class I regulates NMDAR single-channel properties, we estimated NMDAR unitary current using non-stationary noise analysis (NSNA). This method, unlike conventional single-channel recording, allows direct estimation of the behavior of synaptically-localized receptors. NSNA of evoked currents shows that NMDAR unitary currents are significantly enhanced in MHC class I-deficient neurons relative to WT. This single-channel enhancement is also only observed at depolarized membrane potentials, and is sufficient to explain the increase in whole-cell current in MHC class I-deficient animals. MHC class I does not form a stable macromolecular complex with the obligatory GluN1 subunit in brain lysates, suggesting that MHC class I affects NMDAR single-channel properties through one or more signaling

intermediates. Ongoing studies are exploring the effects of MHC class I on post-translational modification of GluN2 subunits, a possible source of changes in unitary currents. Thus MHC class I is a voltage-dependent brake on NMDAR single-channel currents at the synapse. These results clarify a mechanism by which changes in MHC class I expression, like those seen with brain injury, stroke, infection, and aging, might affect the risk of NMDAR-mediated excitotoxicity.

Disclosures: C.M. Tyler: None. **M. Chacon:** None. **J.I. Aguilar:** None. **D.H. Perlman:** None. **L. Fourgeaud:** None. **L.M. Boulanger:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.22/D17

Topic: B.02. Ligand Gated Ion Channels

Support: SUNY REACH

NIH RO1 NS052669-07

Title: Charged residues external to the NMDA receptor gate control gating and conductance

Authors: *B. A. MAKI[1], G. K. POPESCU[2];
[1]Biochemistry, Neurosci., Univ. At Buffalo, SUNY, Buffalo, NY; [2]Biochem., SUNY Buffalo, Buffalo, NY

Abstract: Critical to N-methyl-D-aspartate receptor (NMDAR) functions are the receptor's high Ca2+ permeability, high unitary conductance and characteristically slow kinetics. So far, two molecular determinants of Ca2+ permeability have been identified, both of which are located on the obligatory GluN1 subunit: 1) the asparagine residue situated at the narrow constriction of the channel pore, and 2) a cluster of charged residues within the DRPEER motif, which is located just extracellular to the agonist controlled gate. In the related AMPA receptors, residues located in this latter region control receptor activation and desensitization kinetics. To investigate whether these residues also control single-channel properties of NMDARs, we examined cell-attached currents recorded from GluN1/GluN2A receptors that had single alanine substituted for the charged residues within the DRPEER region. Receptors with alanine substitutions at negatively charged residues (D640A, E643A and E644A) had shorter mean open times (MOTs), whereas R641A, had a ~2-fold longer MOT. Surprisingly, these mutants also had altered conductance and this correlated positively (R2 = 0.9462) with changes in open durations.

Further, we ascertained that the effects on both conductance and gating were conferred by charge ablation, since isosteric mutations (D640N, R641Q) resulted in phenotypes similar to the alanine mutants, whereas introducing charged residues that differed in size (D640E, R641K) produced currents that were indistinguishable from wild-type traces. These results demonstrate that in addition to controlling Ca2+ permeability, residues within the DRPEER also control channel conductance and receptor gating properties. In addition, the correlation we uncovered between open durations and conductance may help investigations into the mechanisms that govern properties of NMDARs that are critical to normal synaptic development and plasticity but also with glutamate excitotoxicity.

Disclosures: B.A. Maki: None. **G.K. Popescu:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.23/D18

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant R01 MH045817

NIH Grant T32 NS073548

Title: Synaptic-like glutamate applications reveal NMDA receptor subtype-dependent inhibition by memantine and ketamine

Authors: *N. G. GLASGOW, J. W. JOHNSON;
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Abstract: NMDA receptors (NMDARs) are tetrameric ionotropic glutamate (glu) receptors usually composed of 2 GluN2 subunits, of which there are 4 types (A-D), and 2 GluN1 subunits. NMDAR subtype is defined by the GluN2 subunits present (e.g. GluN1/2A receptors contain GluN1 and GluN2A subunits). NMDAR properties such as maximal Popen and deactivation kinetics vary by NMDAR subtype. NMDARs are found at most vertebrate excitatory synapses as well as extrasynaptically and are essential to the normal function of the nervous system. Inhibition of NMDARs by open channel blockers has broad potential for treatment of pathological conditions: for example, memantine is approved for treatment of Alzheimer's disease, and ketamine has shown promise in treatment of both depression in patients resistant to other treatments and of neuropathic pain in preclinical models. Although memantine and ketamine share similar kinetics and IC50s at NMDARs, their clinical effects vary significantly,

suggesting that their mechanisms of NMDAR inhibition may differ in ways that are not yet well understood. Previous studies have shown that during long applications of glu, neither memantine nor ketamine shows selectivity between GluN1/2A and GluN1/2B receptors, which contain the subunits thought to predominate at synaptic sites in cortex. However, it was recently demonstrated in neuronal cultures that memantine inhibits synaptic NMDAR currents less effectively than currents activated by long agonist applications (Xia et al., 2010), suggesting that duration of agonist exposure may influence inhibition by memantine and perhaps inhibition by open channel blockers in general. To examine this hypothesis, we measured inhibition by memantine and ketamine as a function of glu application duration and of NMDAR subtype. We performed whole-cell recordings from tsA201 cells transfected to express either GluN1/2A or GluN1/2B receptors, and simulated synaptic glu release using a fast perfusion system optimized to apply glu to lifted cells for ~5 ms. We compared inhibition by memantine and ketamine of synaptic-like glu applications and of long (> 10 s) glu applications. We found that memantine inhibits synaptic-like GluN1/2A, but not GluN1/2B, receptor currents less effectively than responses to long applications. Ketamine also inhibits synaptic-like GluN1/2A receptor currents less effectively than responses to long applications, but inhibits synaptic-like GluN1/2B receptor currents more effectively than responses to long applications. Thus, synaptic-like applications of glu reveal a blocker- and NMDAR subtype-dependence not observed with long glu applications.

Disclosures: N.G. Glasgow: None. **J.W. Johnson:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.24/D19

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant AA015203-06A1

Title: Sites of alcohol action at the GluN1/GluN2B NMDA receptor M3-M4 domain intersubunit interfaces

Authors: Y. ZHAO, M. WU, H. REN, *R. W. PEOPLES;
Biomed. Sci., Marquette Univ., MILWAUKEE, WI

Abstract: The N-methyl-D-aspartate (NMDA) receptor has been shown to be one of the most important target sites of alcohol in the central nervous system. We and others have identified positions in the third and fourth membrane-associated (M) domains of both GluN1 and GluN2A subunits that influence alcohol sensitivity. In the structural model of the NMDA receptor based

upon the related GluA2 receptor, the outward face of the M3 domain of one subunit type is oriented toward the M4 domain of the other subunit type. We recently reported that four pairs of alcohol-sensitive amino acid positions in GluN1/GluN2A NMDA receptors interact at the M3-M4 intersubunit interfaces with respect to alcohol sensitivity and receptor kinetics. Because a number of studies point to a major role for the GluN2B subunit in the action of alcohol in the brain, in the present study we used site-directed mutagenesis and electrophysiological patch-clamp recording in transfected cells to investigate the sensitivity of cognate positions in the GluN2B subunit, as well as interactions between positions in the M3 and M4 domains of the GluN1 and GluN2B subunits affecting ethanol inhibition. Although the M3 and M4 domains of GluN2A and GluN2B are highly conserved, only one of four positions in GluN2B, F637, corresponding to alcohol-sensitive positions in GluN2A exhibited altered ethanol IC_{50} values in substitution mutants. However, we observed interactions with respect to ethanol sensitivity among three out of four pairs of positions (G638/M824, F639/L825, and M818/F637 in GluN1/GluN2B), even when single substitution mutations at one of the two positions in a pair (GluN1 M818; GluN2B M824, L825) had no effect on alcohol sensitivity. These results support the existence of sites of alcohol action formed by clusters of positions at the M3-M4 domain intersubunit interfaces of GluN1/GluN2B NMDA receptors, although they appear to differ from the corresponding sites in GluN1/GluN2A receptors reported previously.

Disclosures: Y. Zhao: None. **M. Wu:** None. **H. Ren:** None. **R.W. Peoples:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.25/D20

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant MH57683

Title: Extrasynaptic NMDA receptors on fast-spiking prefrontal cortical interneurons

Authors: *E. M. LEWIS[1], P. O'DONNELL[2];
[1]Anat. & Neurobio., Univ. of MD Baltimore, Baltimore, MD; [2]Anat. & Neurobio., Univ. of MD, Baltimore, Baltimore, MD

Abstract: Disinhibited cortical circuits are central to current views of schizophrenia pathophysiology. Non-competing NMDA receptor antagonists, known to be psychotomimetic in adults, have been proposed to exert their effect primarily by blocking receptors on fast-spiking interneurons (FSI) yielding increased pyramidal cell activity. Reductions in NMDA signaling

early in development, either globally using pharmacological approaches or specifically in parvalbumin (PV) positive interneurons have been shown to lead to behavioral and electrophysiological phenotypes resembling phenomena observed in schizophrenia patients (Belforte et. al, 2010). However, it has been recently demonstrated that the contribution of NMDA receptor activation to excitatory postsynaptic potentials (EPSP) and currents (EPSC) in FSI is minimal compared to that of pyramidal cells (Rotaru et. al, 2011), calling into question the role that NMDA receptors play in FSI physiology within the adult cortex. Interestingly, cortical FSI show a significant tonic NMDA current in vitro (Povysheva and Johnson, 2012), suggesting that while synaptic events onto FSI might not have a substantial NMDA component, glutamate could modulate FSI activity via extrasynaptic NMDA receptors. We tested whether FSI express functional NMDA receptors in the adult rat medial prefrontal cortex (mPFC) using whole-cell recordings to measure changes in excitability in response to bath application of NMDA and found that NMDA leads to an increase in FSI excitability which is accompanied by a slight depolarization. These responses were not due to activation of other cell types and glutamate release, as AMPA receptors were blocked by CNQX throughout each recording. Given that astrocytic glutamate transporters typically keep glutamate well confined to the synaptic cleft, we tested for the presence of extrasynaptic NMDA receptors by recording evoked synaptic responses in FSI in the presence and absence of the glutamate reuptake inhibitor DL-threo-β-Benzyloxyaspartic acid (TBOA). We found that synaptic responses acquired a prolonged NMDA component during TBOA application suggesting the presence of extrasynaptic NMDA receptors. Our results indicate that even in the absence of a significant synaptic NMDA component, extrasynaptic NDMA receptor activation is likely to play a meaningful role in regulating the activity of fast-spiking interneurons.

Disclosures: E.M. Lewis: None. **P. O'Donnell:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.26/D21

Topic: B.02. Ligand Gated Ion Channels

Support: 1R01GM098089 - 01A1

Title: Probing nmda receptor activation dynamics by combined patch-clamp single-molecule imaging microscopy

Authors: *H. LU, D. K. SASMAL;
Dept. of Chem., Bowling Green State Univ., Bowling Green, OH

Abstract: We have studied the NMDA receptor dynamics in Cho cells and neuron cells, using our newly developed combining real-time single-molecule fluorescence imaging with real-time single-channel electric current recording. We were able to probe cell electrophysiological responses and signals under ligand binding and membrane depolarization, and obtain an understanding the dynamics and mechanism of NMDA receptors at the molecular level. The kinetic behavior of ion channel proteins is regulated by subtle conformational changes that are often difficult to characterize by conventional ensemble-averaged static structure analysis and by interpretations of ion-channel electrophysiological measurements. We have analyzed and identified that subtle structural dynamics of ion channels play an important role in regulating channel function and selectivity, especially sensitive to the protein-lipid interactions and the peptide domain solvation dynamics. Using our unique approaches, we have obtained an understanding of how NMDA ion-channel activities are regulated by the conformational change and protein-membrane interaction dynamics. A new NMDA activation model has been postulated based on our experimental results.

Disclosures: H. Lu: None. **D.K. Sasmal:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.27/D22

Topic: B.02. Ligand Gated Ion Channels

Support: CIHR Grants (MOP-38028, MOP-79428)

CAS

CRC

Savoy Foundation

Kirk Weber Award

Title: Tranexamic acid inhibits N-methyl-D-aspartate receptors in the hippocampus

Authors: *I. LECKER[1], D.-S. WANG[2], D. MAZER[4,3,2], B. A. ORSER[5,3,2];
[2]Physiol., [3]Anesthesia, [1]Univ. of Toronto, Toronto, ON, Canada; [4]Anesthesia, St. Michael's

Hospital, Keenan Res. Ctr. in the Li Ka Shing Knowledge Inst., Toronto, ON, Canada; [5]Anesthesia, Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstract: Background: Tranexamic acid (TXA) is an antifibrinolytic drug widely used to prevent excessive blood loss during surgery. We have previously shown that TXA is a competitive antagonist of glycine receptors. Glycine is a co-agonist of N-methyl-D-aspartate receptors (NMDARs). Therefore, we hypothesized that TXA can inhibit NMDARs by interacting with the glycine binding site
Methods: Experiments were approved by the local ethics review committee. Electrophysiological data was collected from primary cultures of hippocampal neurons prepared from Swiss White embryonic mice. Whole-cell voltage clamp techniques were used to record NMDA-evoked currents in the absence and presence of TXA. Low concentrations of glycine (0.3 - 3 μM) were added to the extracellular solution. All data are expressed as mean ± SEM.
Results: TXA inhibited NMDARs in a dose-dependent manner. The threshold and concentration half maximal inhibitory concentration were 3 mM and 35.5 ± 4.1 mM, respectively. We also found that increasing the extracellular glycine concentrations enhanced the percentage of NMDAR blockade by TXA. The percent inhibition of NMDARs by TXA in the presence of low (0.3 μM) and high (3 μM) concentrations was 21.4 ± 4.9 % and 38.8 ± 5.7 %, respectively.
Conclusion: This is the first evidence showing that TXA inhibits NMDARs potentially by acting as a non-competitive antagonist.

Disclosures: I. Lecker: None. **D. Wang:** None. **D. Mazer:** None. **B.A. Orser:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.28/D23

Topic: B.02. Ligand Gated Ion Channels

Support: Swiss NSF Ambizione grant PZ00P3_136761/1

Title: Time-of-day dependent changes in NMDAR subunits and effects on synaptic plasticity at intrathalamic connections

Authors: *S. ASTORI, A. LUTHI;
DNF, Univ. of Lausanne, Lausanne, Switzerland

Abstract: The subunit composition of synaptic NMDA receptors (NMDARs) remains modifiable throughout adulthood in response to sensory experience and learning. GluN2 subunits

are also modulated by arousal and sleep-promoting agents in different brain areas, and in particular GluN2A-NMDARs at hippocampal synapses are augmented by mild sleep deprivation and render synaptic plasticity susceptible to sleep loss (Longordo et al., 2009). NMDARs are also expressed within thalamic circuits that participate in thalamocortical rhythms apparent in the sleep electroencephalogram, but NMDAR function at intrathalamic synapses remains poorly understood. Here, we examined NMDAR-mediated transmission in the intrathalamic network and tested the GluN2-subunit content and its contribution to synaptic plasticity.
We studied glutamatergic synapses formed by thalamocortical axons on neurons of the nucleus Reticularis thalami (nRt) by means of patch-clamp electrophysiology in acute slices from 3-week-old mice sacrificed at different times of day. Mice were previously entrained to a 12h-12h light-dark cycle with lights on at 7 AM. The pharmacological profile of evoked NMDAR currents indicated an accumulation of GluN2A-NMDARs at the end of the dark phase, i.e. after the active period of the mice. In slices prepared during the last hour before light onset (6-7 AM), NMDAR currents were almost two-fold more sensitive to the GluN2A-preferring blocker NVP-AAM077 (50nM; 38.7±4.3% reduction, n=7) as compared to the first half (23.4±4.1%, n=8, $p<0.05$) and to the second half (21.1±1.8%, n=6, $p<0.01$) of the light phase. NMDA/AMPA ratios were comparable at the three time points (6-7 AM: 0.27±0.04; 11-12 AM: 0.29±0.05; 6-7 PM: 0.30±0.04).
We previously showed that NMDARs promote synaptic plasticity at thalamo-nRt synapses (Astori and Lüthi, 2013). In slices prepared during light phase (11-12 AM), the pairing of synaptic stimulation with repetitive low-threshold nRt bursting at frequencies typical for slow-wave sleep (1Hz) elicited long-term potentiation (44.8±9.9%, n=14, $p<0.01$). Synaptic changes were prevented by the GluN2B-antagonist CP101,606 (10µM; -0.7±2.9%, n=9, $p>0.05$). This finding was consistent with a major contribution of GluN2B to synaptic NMDAR currents during the resting phase (11-12 AM: 65.9±3.6% reduction with CP101,606, n=8, $p<0.05$), while GluN2A-NMDARs appeared to play a minor role. Our data suggest that GluN2B-NMDARs are dominating intrathalamic plasticity, with a time-of-day-dependent additional contribution of GluN2A-NMDARs that remains to be further defined.

Disclosures: S. Astori: None. **A. Luthi:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.29/D24

Topic: B.02. Ligand Gated Ion Channels

Title: Comparison of the *Ex vivi* receptor occupancy profile of ketamine to several NMDA receptor antagonists in mouse hippocampus

Authors: *B. LORD[1], C. WINTMOLDERS[2], X. LANGLOIS[3], L. NGUYEN[4], P. BONAVENTURE[4];
[1]Janssen PRD, L.L.C, SAN DIEGO, CA; [2]Janssen PRD, L.L.C, Beerse, Belgium; [3]Janssen PRD, L.L.C., Beerse, Belgium; [4]Janssen PRD, L.L.C., San Diego, CA

Abstract: NMDA receptor antagonists, particularly these targeting the NR2B subunit are of therapeutic interest for the treatment of severe mood disorders. The receptor occupancy profiles of several NMDA receptor antagonists (30 mg/kg, s.c.) were compared in mouse hippocampus by *ex vivo* autoradiography using [^{3}H]MK-801, a non-selective channel blocker and [^{3}H]ifenprodil a selective NR2B antagonist. Subcutaneous administration of ketamine and memantine (inhibited [^{3}H]MK-801 but not [^{3}H]ifenprodil binding in mouse hippocampus. Ketamine reached maximal occupancy of [^{3}H]MK-801 binding sites after 15 min and rapidly cleared from the brain with no significant level of occupancy measured at the 1 h time point. Memantine significantly occupied [^{3}H]MK-801 binding sites throughout the 6 h time course. The selective NR2B antagonist CP101, and Ro 25-6981 inhibited [^{3}H]ifenprodil but not [^{3}H]MK-801 binding and significant levels of occupancy (above 50%) were measured throughout the 6 h time course. These data highlight the unique quick pulse target engagement profile of ketamine compared to other NMDA receptor antagonists.

Disclosures: B. Lord: None. **C. Wintmolders:** None. **X. Langlois:** None. **L. Nguyen:** None. **P. Bonaventure:** None.

Poster

514. **NMDA Receptor Trafficking and Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 514.30/D25-DP2

Topic: B.02. Ligand Gated Ion Channels

Title: Amyloid-beta and agonist induce ion-flow independent conformational changes in the cytoplasmic domain of nmda receptors as monitored by fret-flim

Authors: *K. B. DORE, J. AOW, R. MALINOW;
Neurosciences, UCSD, La Jolla, CA

Abstract: Transmembrane receptors are well known to transduce extracellular ligand binding to intracellular signaling by transmitting transmembrane conformational changes. Whether ion-

channels perform the same task is not well established. Here we show that both ligand binding to the NMDA receptor and amyloid-beta application can drive a conformational change in the cytoplasmic domain of the NMDA receptor. We used GluN1 subunits tagged at the c-terminus with either GFP or mCherry as a sensor to measure these conformational changes with FRET-FLIM. Amyloid-beta produced an increase in FRET between the GluN1 c-tails that was dependent on the EphB2 receptor. However application of the specific agonist NMDA produced a reduction in FRET, meaning that the c-tails were driven farther apart upon direct agonist binding. Interestingly, both processes required ligand binding to the GluN2 subunit, and occurred despite blockade of NMDA receptor ion passage or of glycine binding to GluN1.

Disclosures: K.B. Dore: None. **J. Aow:** None. **R. Malinow:** None.**Poster**

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.01/D26

Topic: B.02. Ligand Gated Ion Channels

Support: HL 105703

Title: NADA activates CB1 and TRPV1 independently to discretely regulate separate glutamate vesicle pools in the solitary tract nucleus

Authors: *J. A. FAWLEY, M. E. HOFMANN, M. C. ANDRESEN;
Dept Physiol & Pharmacol, Oregon Hlth. & Sci. Univ., PORTLAND, OR

Abstract: Endogenous lipid signals activate both the G protein-coupled cannabinoid receptor 1 (CB1) and the non-selective cation channel Transient Receptor Potential Vanilloid Type 1 receptor (TRPV1). This presents the possibility for activation of CB1 and TRPV1 by a single neuromodulator and for signaling crossover within the terminals of solitary tract (ST) afferents that express both receptors. ST afferents are differentiated by the presence of TRPV1 at C-fibers or its absence at A-fibers. While both afferent types have similar ready-releasable vesicle pools that generate synchronous glutamate release, only TRPV1+ afferents have an additional, TRPV1-operated vesicle pool that that releases both asynchronous and temperature-evoked glutamate. Here we tested whether NADA (5-10 μM), an endogenous lipid agonist with a high affinity for CB1 and TRPV1, can modulate the different forms of vesicular glutamate release from ST afferents. Shocks to the ST evoked synchronous glutamatergic EPSCs (ST-eEPSCs) with a jitter (SD of the latency) < 200 μs. Asynchronous release after ST-eEPSCs, or the lack thereof, identified TRPV1+ or TRPV1- synapses, respectively. Basal sEPSCs were measured prior to ST-eEPSCs. Repeated bath temperature ramps from 32-36° C reversibly increased the

rate of TRPV1-operated sEPSCs. NADA depressed ST-eEPSC amplitudes equally from CB1+/TRPV1+ and CB1+/TRPV1- afferents (66 and 67% ctrl, respectively) indicating comparable action at the readily releasable pool. Despite substantial reduction of ST-eEPSCs, NADA did not change the rate of basal sEPSCs from CB1+/TRPV1- afferents (99% ctrl) but enhanced both basal (140%) and temperature-evoked sEPSCs (135%) from TRPV1+ afferents with CB1. Interestingly, in TRPV1+ afferents lacking CB1, NADA only enhanced TRPV1-operated glutamate release, indicating that there is an additional, potentiating action at the TRPV1-operated pool. Therefore despite co-localization in most ST afferents, activation of CB1 had no effect on TRPV1 function and vice versa. Together, these data demonstrate that CB1 and TRPV1 discretely alter the different, independently regulated vesicle pools from ST afferents.

Disclosures: J.A. Fawley: None. **M.E. Hofmann:** None. **M.C. Andresen:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.02/D27

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant 1F32HL112419-01

NIH Grant 5R01HL10570303

Title: TRPV1 antagonists block capsaicin but not temperature activation of TRPV1 at 2nd order neurons in the nucleus of the solitary tract

Authors: *M. HOFMANN, J. A. FAWLEY, M. C. ANDRESEN;
OHSU, Portland, OR

Abstract: In the nucleus of the solitary tract (NTS), most 2nd order neurons receive primary sensory cranial afferents that express the transient receptor potential vanilloid type I (TRPV1) receptor. Low pH (<5), high temperature (>43°C), or ligands activate TRPV1, which results in the influx of calcium through its non-selective cation pore. This increase in intraterminal calcium releases glutamate to generate EPSCs. We previously demonstrated that physiological temperatures (32-36 °C) activate TRPV1 to trigger increases in spontaneous EPSCs in the NTS. Here we investigated whether TRPV1 antagonists blocked temperature and capsaicin responses using patch recordings. In horizontal brain stem slices, solitary tract afferent stimulation recruited monosynaptic inputs and most cells responded to increased temperature and application of the TRPV1 agonist capsaicin with increased spontaneous EPSCs, i.e. they were TRPV1+. In

these cells, ramp increases in bath temperature from 32-36 °C over 3 minutes increased spontaneous EPSC rate but did not alter amplitudes. The TRPV1 antagonists A784168 and JNJ 17203212 attenuated responses to capsaicin but failed to inhibit temperature responses. In contrast, capsazepine and SB452533 significantly increased the temperature-induced increase in spontaneous EPSC rates but blocked responses to capsaicin. These results suggest that capsazepine and SB452533 may act as partial agonists at TRPV1 in the NTS. Capsaicin also blocks solitary tract evoked EPSCs and all four compounds were effective at preventing this action. These results demonstrate that each of these TRPV1 antagonists appear to effectively block the ligand activation site of TRPV1 but not the thermal activation site. The published profiles of several of these antagonists suggest that they effectively blocked multiple activation sites of TRPV1 (pH, heat, ligand) in heterologous screening systems. Our studies suggest that either central TRPV1 of cranial visceral afferents significantly varies from peripheral, often somatic afferent TRPV1 or solitary tract afferents are substantially sensitized to thermal inputs. For therapeutic use of TRPV1 antagonists, the presence of sensitive TRPV1 receptors coupled to neurotransmitter release in the NTS may present a potentially important focus for broad autonomic sequelae.

Disclosures: M. Hofmann: None. **J.A. Fawley:** None. **M.C. Andresen:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.03/D28

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant HL65701

Title: Propofol restores TRPV1 sensitivity via a TRPA1-, NOS-dependent activation of PKCε in sensory neurons

Authors: P. SINHA ROY, B. PRUDNER, S. SINHA, *D. S. DAMRON;
Biol. Sci., Kent State Univ., Kent, OH

Abstract: Background: The intravenous anesthetic, propofol, restores the sensitivity of transient receptor potential vanilloid channel subtype-1 (TRPV1) receptors via a protein kinase C epsilon (PKCε)-dependent phosphorylation of the receptor. Moreover, propofol restores TRPV1 sensitivity via a transient receptor potential ankyrin channel subtype-1 (TRPA1)-dependent pathway. Our objectives were to determine the extent to which PKCε is involved in mediating the TRPA1-dependent restoration of TRPV1 sensitivity and the mechanism by which TRPA1

stimulation causes PKCε activation.
Methods: Lumbar mouse dorsal root ganglion (DRG) neurons were isolated and cultured for 24 hrs. F-11 cells were transfected with complimentary DNA (cDNA) for TRPV1 only or both TRPV1 and TRPA1. Intracellular Ca^{2+} concentration was measured in individual cells via fluorescence microscopy. Following TRPV1 de-sensitization with capsaicin (100 nM), cells were treated with various interventions prior to subsequent reapplication of capsaicin. Immuno-blot analysis of the total and phosphorylated forms of PKC and TRPV1 was also performed.
Results: In DRG neurons or F-11 cells containing both TRPV1 and TRPA1, PKCε inhibition (V1-2) prevented the propofol- and allylisothiocyanate (AITC) -induced restoration of TRPV1 sensitivity to agonist stimulation. In F-11 cells transfected with TRPV1 only, neither propofol nor AITC induced PKCε or TRPV1 phosphorylation. However, in F-11 cells transfected with both TRPV1 and TRPA1, both propofol and AITC increased phosphorylation of PKCε and TRPV1 that could be blocked with the PKCε inhibitor peptide, V1-2. Moreover, nitric oxide synthase inhibition blocked propofol-and AITC-induced restoration of TRPV1 sensitivity as well as PKCε phosphorylation, and V1-2 prevented the nitric oxide donor, SNAP, from restoring TRPV1 sensitivity.
Conclusion: These data indicate that the AITC- and propofol-induced restoration of TRPV1 sensitivity is mediated by a TRPA1-dependent, nitric oxide synthase-dependent activation of PKCε.

Disclosures: P. Sinha Roy: None. **D.S. Damron:** None. **B. Prudner:** None. **S. Sinha:** None.

Poster

515. TRP Channel Physiology and Pharmacology

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.04/D29

Topic: B.02. Ligand Gated Ion Channels

Support: NRF Grant 2012R1A3A2048834

NRF Grant 2012R1A1A1042191

Title: The pharmacological action of eugenol involves rapid desensitization of TRPA1

Authors: *G. CHUNG, Y. KIM, S.-T. IM, I. JANG, S. OH;
Neurobio. & Oral Physiol., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The structural similarity of eugenol to cinnamaldehyde, an active ligand for transient receptor potential ankyrin 1 (TRPA1), suggests that eugenol might produce its effect via TRPA1

in addition to TRPV1 as we reported previously. In this study, we investigated effect of eugenol on TRPA1 and TRPA1-associated orofacial pain. As the results, eugenol induced robust calcium responses and inward currents in a subset of rat trigeminal ganglion neurons that responded to a specific TRPA1 agonist, allyl isothiocyanate (AITC). The eugenol response was observed in trigeminal ganglion neurons from TRPV1 knockout mice and human embryonic kidney 293 cells that stably express TRPA1, which was inhibited by a TRPA1-specific antagonist HC-030031, but not by a TRPV1 specific antagonist capsazepine. Interestingly, we found that eugenol alleviated formalin-induced orofacial pain, which might be associated with rapid TRPA1 desensitization as revealed in electrophysiological experiments. In conclusion, our results demonstrate that fast desensitization of TRPA1 can account for novel molecular mechanism underlying pharmacological action of eugenol.

Disclosures: G. Chung: None. **Y. Kim:** None. **S. Im:** None. **I. Jang:** None. **S. Oh:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.05/D30

Topic: B.02. Ligand Gated Ion Channels

Support: GACR 305/09/0081

MSMT OP VK CZ.1.07/2.3.00/30.0025

PRVOUK P45

SVV-2010-261 304

GAUK 426311

GAUK 888513

Title: Identification of functional microdomains within the S4-S5 linker of human TRPA1 channel

Authors: A. HYNKOVA[1], K. WITSCHAS[1], V. ZIMA[2], L. SURA[1], I. BARVIK[2], *V. VLACHOVA[1];
[1]Inst. of Physiol. AS CR, Prague, Czech Republic; [2]Inst. of Physics, Charles Univ., Prague, Czech Republic

Abstract: Transient receptor potential ankyrin 1 channel (TRPA1) is a sensory-neuron-specific ion channel that is gated in response to a variety of pungent chemicals, including allyl isothiocyanate and cinnamaldehyde, while it is strongly modulated by permeating Ca^{2+}. In the absence of any agonist, this channel can also be activated by depolarizing voltages. The molecular events transmitting such disparate signals to the central pore gate domain of the channel are unknown.
The molecular architecture of the transmembrane region of TRPA1 is thought to be potentially analogous to the large family of voltage-dependent potassium channels, in which the S4-S5 linkers, cytosolic loops connecting the S4 and S5 membrane spanning domains, mediate signal transduction and stabilize conformations associated with gating states. To address the functional role of the putative S4-S5 linker (Q851-F859) in TRPA1 channel, we performed mutagenesis studies targeting the highly conserved charged residues R852 and E854, and also E864 and K868 from the adjacent S5. Activation parameters including sensitivity to 100 µM cinnamaldehyde, the extents of Ca^{2+}-dependent potentiation and inactivation, and the steady-state voltage dependence of activation were explored for each mutant using whole-cell and single-channel recordings. The previously described gain-of-function mutation associated with familial episodic pain syndrome N855S (Kremeyer et al, 2010, Neuron 66: 671-680) was also examined for comparison. The effects of mutations at several residues had complex phenotypes indicating an unequal contribution of these residues to different modalities of TRPA1 activation and, therefore, apparently contradicting the predicted generic role of the S4-S5 linker in „simply“ coupling the S1-S4 module to the S5-S6 activation gate. Remarkably, the N855R mutation resulted in an increased sensitivity to cinnamaldehyde and a faster Ca^{2+}-induced inactivation kinetics without changing Ca^{2+} potentiation or steady-state voltage dependency. On the other hand, the charge reversal mutation R852E exhibited a gain-of-function when activated by cinnamaldehyde but impaired the potentiating effect of Ca^{2+}. Thus, the current work indicates that the S4-S5 linker acts as a signal integrator and a modality-selective transducer that is differentially involved in distinct sets of interactions that regulate the polymodal gating of the TRPA1 channel.
This work was supported by GACR 305/09/0081, MSMT OP VK CZ.1.07/2.3.00/30.0025, PRVOUK P45, SVV-2010-261 304, GAUK 426311 and 888513.

Disclosures: A. Hynkova: None. **K. Witschas:** None. **L. Sura:** None. **V. Vlachova:** None. **V. Zima:** None. **I. Barvik:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.06/D31

Topic: B.02. Ligand Gated Ion Channels

Support: GACR 301/10/1159

GACR 207/11/0717

GACR P304/12/G069

Title: Multiple ligand binding sites within intracellular N-terminus of TRPM1

Authors: *M. JIRKU[1], K. BOUSOVA[1], L. BUMBA[2], J. TEISINGER[1];
[1]Inst. of Physiol. ASCR, V.v.I., Prague, Czech Republic; [2]Inst. of Microbiology ASCR, v.v.i., Prague, Czech Republic

Abstract: Melastatin channel (MLSN) or TRPM1 (transient receptor potential melastatin 1) is the founding member of the subfamily of TRPM ion channels belonging to the superfamily of TRP channels. It is assumed that TRPM1 channel has six transmembrane domains with a pore domain between the fifth and the sixth segments. Intracellularly located N- and C-tails are responsible for regulation of TRP channels, which carry binding sites for signal molecules like calmodulin (CaM) or S100A1. [1-3]
TRPM1 is expressed in human melanocytes and bipolar cells in retina and participates in processes connected to vision. Mutations of TRPM1 gene are associated with congenital stationary night blindness in humans. [4-6] There is a scarcity of structural and functional properties of TRPM1 channel.
Two independent CaM /S100A1 binding sites on the intracellular N-terminus of rat TRPM1 were identified. Using bioinformatic approach we found Ca2+-dependent CaM /S100A1 binding sites in regions L242-E344 and A451-N566 corresponding to the consensus CaM binding motif 1-8-14. Several basic and hydrophobic amino acid residues responsible for binding in these regions of TRPM1 to CaM and S100A1 were determined.
Fusion proteins of both TRPM1 domains and appropriate mutants were expressed in E.coli and purified according to the two-step purification protocol. Amino acid sequence was checked by mass spectroscopy. The equilibrium dissociation constants for binding of CaM and S100A1 to TRPM1 fusion proteins and its mutants were estimated using by fluorescence anisotropy measurement and surface plasmon resonance measurement was employed as well. Experimental data also indicate that CaM and S100A1 bind to the same or overlapping binding sites.
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Disclosures: M. Jirku: None. **K. Bousova:** None. **L. Bumba:** None. **J. Teisinger:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.07/D32

Topic: B.02. Ligand Gated Ion Channels

Support: National Research Foundation of Korea(NRF) grant (2012R1A3A2048834) funded by the Korea government(MEST)

Title: Involvement of TRPM2 channels in microglia activation

Authors: *H. JEONG[1], Y. KIM[1], S. JUNG[2], S. OH[1];
[1]Physiol. and Neurobio., Seoul Natl. Univ., SEOUL, Korea, Republic of; [2]Dept. of Pysiology, Hanyang Univ., Seoul, Korea, Republic of

Abstract: Microglia are resident macrophages in the central nervous system which become activated in response to peripheral tissue damage, inflammation, or nerve injury. Various endogenous immunomodulators are generated in the brain and spinal cord under pathological conditions. It has been demonstrated that microglia transform from ramified into ameboid morphology upon microglia activation which is associated with large amounts of Ca2+ influx in microglia. However, underlying mechanism of Ca2+ influx remains unknown. Transient receptor potential melastatin 2 (TRPM2) channel is a nonselective cation channel permeable to Ca2+ that is highly expressed in immune cells such as microglia. TRPM2 is activated by reactive oxidative species (ROS) including intracellular adenosine diphosphate ribose (ADPR), H2O2. Here, we examined the role of TRPM2 in microglia activation using BV-2 cells and murine primary cultured microglia.
Ca2+ imaging data showed that increase of [Ca2+]i in microglia was abolished in the presence of Gd3+, flufenamic acid, a TRPM2 channel blocker, and in Ca2+-free extracellular solution. TRPM2-mediated Ca2+ influx is occurred in wild-type microglia but not in TRPM2-/- microglia. LPS induced up-regulation of phospho-p38, a well known marker of microglia activation, in wild-type mice, which was abolished in TRPM2-/- mice. Furthermore, TRPM2 expression is increased following LPS treatment in wild-type microglia but not in TRPM2-/- mice. Our results suggest that TRPM2-mediated Ca2+ signaling leads to microglia activation.

Disclosures: H. Jeong: None. **Y. Kim:** None. **S. Jung:** None. **S. Oh:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.08/D33

Topic: B.02. Ligand Gated Ion Channels

Support: NIH grant HL088520

Title: Kinetics of rapid covalent modification by electrophilic activators of TRPA1

Authors: *P. K. BAHIA, T. E. TAYLOR-CLARK;
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Abstract: Transient Receptor Potential Ankyrin 1 is a non-selective cation channel expressed largely on nociceptive sensory nerves. TRPA1 is activated by electrophilic compounds through covalent modification of select cysteine (Cys) residues on the intracellular N-terminus, and this mechanism is thought to contribute to nociceptor activation during oxidative stress. We analyzed the rate of electrophile-induced TRPA1 currents and calculated that TRPA1 is activated at rates > 1000 M-1s-1. Such rates are substantially faster than the electrophilic covalent modification of Cys on other proteins (<3 M-1s-1). We hypothesized that TRPA1 has unique properties that facilitate rapid electrophilic covalent modification of relevant Cys and thus are likely crucial for TRPA1 to act as a nociceptive transduction mechanism. We have expressed human TRPA1 with a C-terminal V5/poly-His tag in HEK293 cells. Using a pulse-chase experimental design with a fluorescently tagged electrophilic ligand (e.g. BODIPY-iodoacetamide, fluorescein isothiocyanate) we have studied the kinetics of TRPA1 covalent modification following immunoprecipitation. As expected human TRPA1 is rapidly bound by tagged electrophiles at rates >1000 M-1s-1. In comparison we found no significant binding of the ion channel Kv1.4. Surprisingly, the rattlesnake ortholog of TRPA1, which is functionally insensitive to electrophiles, reacted rapidly with electrophiles. In further studies we found that TRPA1 binding of electrophiles is not modified by intracellular polyphosphates (required for TRPA1 currents), and binding of BODIPY-iodoacetamide is irreversible over 40 minutes. By comparing functional TRPA1 responses with quantitative analysis of electrophilic binding, this study allows for novel insights into the activation mechanism of TRPA1.

Disclosures: P.K. Bahia: None. **T.E. Taylor-Clark:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.09/D34

Topic: B.02. Ligand Gated Ion Channels

Title: TRPV1 and TRPV2 are differentially involved in oral persistent pain associated with mucosal injury

Authors: *K. URATA[1], M. SHINODA[2], J. LEE[1], N. GIONHAKU[1], K. IWATA[2];
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Abstract: The wearing of removable denture frequency causes mucosal injury. It is well known that mucosal injury causes severe persistent pain in the oral structures, which many patients suffer from. Previous studies have reported that transient receptor potential vanilloid (TRPV)1 is involved in oral persistent pain following mucosal injury. On the other hand, the involvement of TRPV2 in oral persistent pain remains unclear. It is important to know mechanisms underlying persistent oral pain associated with mucosal injury, to develop the appropriate prosthetic treatment for edentulous patients. In this study, we determined if TRPV1 or TRPV2 was involved in altered mechanical and heat sensitivity in the buccal mucosa (intra-oral tissue) or whisker pad skin (extra-oral tissue) following each tissue incision. Male Sprague-Dawley rats underwent a buccal mucosa or whisker pad skin incision (length: 10mm, Depth: 5mm), and the head-withdrawal reflex threshold (HWRT) to mechanical or heat stimulation of the buccal mucosa or whisker pad skin was analyzed. Moreover, the expression of TRPV1 and TRPV2 in trigeminal ganglion (TG) neurons innervating the buccal mucosa or whisker pad skin was examined, and the effect of local administration of TRPV1 or TRPV2 antagonist on mechanical or heat HWRT was tested. The HWRT to mechanical or heat stimulation of the buccal mucosa or whisker pad skin significantly decreased on day 3 after each incision. The number of TRPV1 and TRP2-immunoreactive (IR) TG neurons innervating the buccal mucosa or whisker pad skin significantly increased on day 3 after each tissue incision. The number of TRPV1 or TRPV2- IR TG neurons innervating the buccal mucosa was significantly larger than that of whisker pad skin. Local administration of TRPV2 antagonist caused significant increase in the HWRT to mechanical or heat simulation of buccal mucosa or whisker pad skin. On the other hand, TRPV1 antagonist administration induced significant increase in HWRT to heat but not mechanical stimulation of the buccal mucosa, and significant increase in HWRT to heat stimulation of buccal mucosa and whisker pad skin.

Disclosures: K. Urata: None. **M. Shinoda:** None. **N. Gionhaku:** None. **K. Iwata:** None. **J. Lee:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.10/D35

Topic: B.02. Ligand Gated Ion Channels

Support: Aix-Marseille University

Title: PKD2L1 channels modulate medullar CSF-contacting neurons excitability by detecting changes in extracellular medium composition

Authors: A. ORTS-DEL'IMMAGINE, J. TROUSLARD, V. TILLEMENT, C. TARDIVEL, *N. WANAVERBECQ;
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Abstract: In vertebrates, cerebrospinal fluid contacting neurons (CSF-cN) are present around the ventricular cavities and along the central canal (cc) where they have a peculiar morphology with a projection towards the cc that ends with a protrusion or 'bud' baring cilia. We have recently shown that CSF-cN are present in the dorsal vagal complex (DVC), a major hindbrain structure regulating autonomic functions. There, CSF-cN receive GABA- and Glycinergic synaptic entries and express functional polycystin kidney disease 2-like 1 (PKD2L1) channels. These channels are a subtype of the transient potential receptor (TRP) channels superfamily and their activity is modulated by variations in extracellular pH and osmolarity.
Using immunohistological techniques on adult PKD2L1:EGFP mice, we characterized the distribution and morphology of medullar CSF-cN. We also determined the consequences of PKD2L1 activation on CSF-cN physiology, using whole-cell patch-clamp recordings in brainstem slices obtained from adult wild type and PKD2L1-knock out (KO) mice.
We demonstrate that around the cc, PKD2L1+ CSF-cN are GABAergic with a density and distribution depending on their position along the rostro-caudal axis. These neurons project a dendrite towards the cc and posses a primary cilium on their soma, not on the bud. Further, they express PKD2L1 channels on the somatodendritic compartment but not on the axon.
In wild type mice, CSF-cN, recorded at a holding potential of -80 mV, exhibit a spontaneous unitary current with a low open probability. This activity is increased by extracellular alkalinization or hypo-osmotic shocks but inhibited by acidification. In current-clamp mode, CSF-cN action potentials discharge is enhanced by the increased PKD2L1 channel activity. In

contrast, in PKD2L1-KO litter mate, we did neither observe single channel activity nor a modulation of CSF-cN excitability.
Taken together our results characterize, for the first time, the morphology and phenotype of mice medullar CSF-cN and demonstrate that they express functional PKD2L1 channels. Further, PKD2L1 activity is modulated by changes in the extracellular medium. In turn, an enhanced activity of only few PKD2L1 channels participates in the modulation of CSF-cN excitability. Because CSF-cN are strategically positioned between CSF and parenchyma, they could detect circulating signals through PKD2L1 activation and convey the collected messages to cellular partners. Such a role might be particularly relevant at the level of the DVC a major regulatory site for autonomic functions and should be demonstrated by identifying and characterizing the neuronal network they are involved in.

Disclosures: A. Orts-Del'Immagine: None. **J. Trouslard:** None. **V. Tillement:** None. **C. Tardivel:** None. **N. Wanaverbecq:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.11/D36

Topic: B.02. Ligand Gated Ion Channels

Title: Irritants activate transient receptor potential channels and non-TRP channel receptors in trigeminal chemosensory neurons of mice

Authors: *R. LEHMANN[1], N. SCHOEBEL[1], H. HATT[2], C. VAN THRIEL[1];
[1]Leibniz Res. Ctr. For Working Envrn. and Human Factors, Dortmund, Germany;
[2]Cellphysiology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: In many occupational fields employees are exposed to various harmful chemicals such as solvents and irritants. Trigeminal chemosensory nerve endings that innervate the facial skin, the tongue, and the mucosae of mouth and nose are the first sensors of the adverse effects of irritants. Such chemicals are detected by many different receptors and ion channels present in terminals of trigeminal sensory neurons, among them the important family of transient receptor potential (TRP) cation channels. Stimulation of multimodal trigeminal nerve fibers triggers reflexes like mucus secretion and respiratory depression. As an indicator of sensory irritation, the 50% decrease of respiratory rates (RD50) after inhalational chemical exposure of rodents is used as the most important bioassay (Alarie test). RD50 values have been established for a large number of chemicals, but so far the underlying cellular mechanisms of receptor activation and

signal transduction are unknown for most substances. In order to identify the cellular detection mechanisms of several chemically diverse irritants with known RD50 values, we employed calcium imaging and electrophysiological techniques in vitro. Using calcium imaging experiments, we observed robust and reproducible responses upon stimulation of trigeminal sensory neurons of mice with irritants like acetophenone (RD50 102.0 ppm), isophorone (RD50 27.8 ppm), or hexyl isocyanate (RD50 4.8 ppm). Based on dose-response relationships, we calculated EC50 values for the irritant-induced responses of isolated neurons. Interestingly, the EC50 values correlated with literature RD50 values indicating that tested irritants have similar effects in vitro and in vivo. Beyond the mere description of irritant effects on isolated trigeminal sensory neurons, we investigated the receptive mechanisms. By using specific blockers in calcium imaging experiments, we could show that some irritants activate TRP channels, whereas other irritants appeared to activate non-TRP receptors. These results were confirmed by electrophysiological recordings on heterologously expressed TRP channels. For the classification of chemicals with respect to their potency to elicit sensory irritation it is important to understand more about the underlying cellular mechanisms of receptor activation and signal transduction pathways and to identify differences between chemicals or classes of chemicals.

Disclosures: R. Lehmann: None. **N. Schoebel:** None. **H. Hatt:** None. **C. van Thriel:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.12/D37

Topic: B.02. Ligand Gated Ion Channels

Support: KAKENHI 24111507

KAKENHI 25640003

Brain Science Foundation

Sumitomo Foundation

Takeda Science Foundation

Salt Science Research Foundation No.12C2

Title: TRPV4 is an important transducer that converts brain temperature energy into neuronal electrical excitability

Authors: *K. SHIBASAKI[1], M. TOMINAGA[2], Y. ISHIZAKI[1];
[1]Dept. of Mol. and Cell. Neurobio., Gunma Univ. Grad. Sch. of Med., Maebashi, Japan; [2]Natl. Inst. for Physiol., Okazaki, Japan

Abstract: Physiologicalbrain temperature is an important determinant of brain functions, and it iswell established that changes in brain temperature dynamically influence hippocampal neuronal activity. We previously revealed that the thermo-sensor TRPV4 (activated above 34°C) is activated by physiological brain temperature in hippocampal neurons and thereby controls their excitability *in vitro* (J. Neurosci. 2007, Shibasaki et al.). Here, we examined whether TRPV4 regulates neuronal excitability through its activation by brain temperature *in vivo*. We developed an original device to cool local brain temperature to inactivate TRPV4. The cooling treatment clearly demonstrated that constitutive TRPV4 activation was occurred in mouse brain, and we found that hippocampal theta-frequency electroencephalogram (EEG) activities in TRPV4KO mice during wake periods were significantly reduced compared with those in WT mice. Furthermore, slice patch clamp recordings from dentate gyrus of hippocampus revealed that WT neurons further depolarized compared with TRPV4KO neurons. Depending on the differences of the resting membrane potentials, WT neurons had smaller fEPSPs and higher firing than KO neurons at 35°C (above TRPV4 activation), however, the differences were abolished at 30°C (less than TRPV4 activation). Taken together, for the first time we reveal that TRPV4 is an important translator that converts brain temperature into neuronal excitability in mammals.

Disclosures: K. Shibasaki: None. **M. Tominaga:** None. **Y. Ishizaki:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.13/D38

Topic: B.02. Ligand Gated Ion Channels

Support: MINECO Grant BFU2012-33334

BCG Grant IT764-13

RETICS RD07/0001/2001

L. Reguero holds a Postdoctoral Specialization Contract from the University of the Basque Country UPV/EHU

Title: Immunoelectron localization of the transient receptor potential vanilloid type 1 at inhibitory synapses in the mouse dentate gyrus

Authors: *P. GRANDES, M.-J. CANDUELA, J.-L. MENDIZABAL-ZUBIAGA, L. REGUERO, N. PUENTE;
Basque Country Univ., Leioa, Spain

Abstract: The transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel that acts primarily as pain sensor in the periphery but also modulates neurotransmitter release and synaptic plasticity in the brain. TRPV1 function must lay on its anatomical distribution in the peripheral and central nervous system regions where the channel has physiological roles. However, the anatomical localization of TRPV1 is well established in the periphery, but it is a matter of debate in the brain. We have recently shown that TRPV1 is highly concentrated in postsynaptic dendritic spines to asymmetric perforant path synapses in the outer 2/3 of the dentate molecular layer, being poorly expressed at the excitatory hilar mossy cell synapses in the inner 1/3 of the layer. On the other hand, the TRPV1 distribution at inhibitory synapses in the dentate molecular layer is still an open question.
In order to investigate this, we have used TRPV1 antibodies combined with a highly sensitive pre-embedding immunogold method for high resolution electron microscopy. TRPV1 immunoparticles were observed in dentate granule cell dendrites receiving symmetric synapses. The silver-intensified gold particles were mostly confined to postsynaptic membranes and were distributed at a relative short distance from the inhibitory synaptic contacts. Importantly, the TRPV1 distribution pattern at inhibitory synapses disappeared in the molecular layer of TRPV1-knockout mice. These findings give additional knowledge regarding to the fine TRPV1 localization obtained with high resolution electron microscopy in the rodent hippocampus.

Disclosures: P. Grandes: None. **M. Canduela:** None. **J. Mendizabal-Zubiaga:** None. **L. Reguero:** None. **N. Puente:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.14/D39

Topic: B.02. Ligand Gated Ion Channels

Title: TRPV1 expression in central histaminergic neurons of rat and mouse

Authors: *R. DE LUCA, A. KERNDER, Y. YANOVSKY, O. A. SERGEEVA;
Inst. of Neuro and Sensory Physiology, Heinrich-Heine Univ. Duesseldorf, Heinrich-Heine Univ. Duesseldorf, Med. Faculty, Neurophysiol., Düsseldorf, Germany

Abstract: The vanilloid receptor 1 (TRPV1) is a nonselective cation channel activated by noxious heat, protons and capsaicin (Tominaga et al., 1998). Recently, it became evident that expression of TRPV1 is not restricted to the primary afferent nociceptors of the dorsal root, trigeminal and nodose ganglia, but also found in CNS (e.g. caudal hypothalamus) and in non-neuronal tissues (Cavanaugh et al., 2011). We have found recently that central histaminergic neurons controlling wakefulness express ASICs (acid-sensing ion channels) and respond to protons with excitation (Yanovsky et al., 2012). We report now that rat histaminergic neurons in the tuberomamillary nucleus (TMN) recorded in slices at 34°C increase firing rate under capsaicin (10µM) to 300±80% (n=4) of control. TRPV1 receptor antagonist capsazepin modifies inward currents in response to pH 6.0 in 30% of acutely isolated rat TMN neurons. With primers spanning fragment of C-terminus (nt 2045-2472, mouse TRPV1 cDNA sequence, NM_001001445), we detect by single-cell RT-PCR TRPV1 transcripts in 20% of rat and in 10% of mouse histaminergic neurons. With other primer sets we detect also the TRPV1 splice variants TRPV1B and Vr.5´sv (AF158248) in some rat neurons. We detect the mouse splice variant TRPV1β in the arcuate nucleus, but not in the TMN region. The majority of mouse histaminergic neurons respond to 10µM capsaicin with a decrease in firing (at 22°C) to 36±7% (12 out of 13 cells), whereas only 25% of the neurons respond to capsaicin 1µM. A subpopulation of cultured histaminergic neurons as well as neurons in slices from tomato-HDC mice show TRPV1 immunoreactivity. The expression of TRPV1 in TMN indicates a role of histaminergic neurons in the control of body temperature and in the systemic response to hypercapnia.
Cavanaugh et al. (2011). J Neurosci, 31, 5067-5077; Tominaga et al (1998) Neuron, 21, 531-543;Yanovsky et al. (2012) Front Syst Neurosci, 6, 23.

Disclosures: R. De Luca: None. **A. Kernder:** None. **Y. Yanovsky:** None. **O.A. Sergeeva:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.15/D40

Topic: B.02. Ligand Gated Ion Channels

Support: GACR 301/10/1159

GACR 207/11/0717

Title: The interactions of the intracellular regions of the TRPM4 channel with calcium binding proteins

Authors: *K. BOUSOVA[1], M. JIRKU[1], L. BUMBA[2], J. TEISINGER[1];
[1]Inst. of Physiology, Acad. of Sci. of the Czech Republic, Prague, Czech Republic; [2]Inst. of Microbiology, Acad. of Sci. of the Czech Republic, Prague, Czech Republic

Abstract: Transient receptor potential channel TRPM4 belongs to the TRP family of non-selective cation channels responsible for entry of mono and bivalent cations (K+, Na+, Ca2+, Mg2+) into the cell. TRP channels occur in membranes and participate on many processes e.g. sensitivity to warm and cold, taste, pressure, light and pain. [1, 2,] Especially the TRPM4 participates in processes ongoing in neurons, cardiomyocytes, pancreatic cells, etc. [1, 3, 4] Activity of the TRPM4 channel could be modulated by intracellular ligands, like calcium binding proteins calmodulin (CaM) and S100A1. [5]
There were characterized one CaM/S100A1 binding site in TRPM4 N-terminal region (NT) S583-A668, and another one independent CaM/S100A1 binding site in C-terminus domain (CT) V1050-S1114. The results from the previous experiments show that CaM and S100A1 compete for the same binding sites. [5] Fusion proteins of the corresponding lengths were expressed in E.coli and purified by HPLC. Sequences of the proteins were verified by Mass Spectrometry. To characterize CaM/S100A1 binding site on intracellular termini regions of the TRPM4 fluorescence anisotropy and surface plasmon resonance measurements were used. Several positively charged residues were identified to be responsible for binding of CaM and S100A1 within the TRPM4-CT S1050-A1114 and the TRPM4-NT S583-A668 domains. The binding of both domains to CaM and S100A1 respectively is Ca2+ dependent.
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[5] Holakovska B., Grycova L., Jirku M., Sulc M., Bumba L., Teisinger J., J. Biol. Chem., 287 (2012), 16645-55.

Disclosures: K. Bousova: None. **M. Jirku:** None. **L. Bumba:** None. **J. Teisinger:** None.

Poster

515. **TRP Channel Physiology and Pharmacology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 515.16/D41

Topic: B.02. Ligand Gated Ion Channels

Support: CIHR Grant MOP111211

Title: Pharmacological characterization and modulation of a cation channel in *Aplysia* bag cell neurons

Authors: *R. M. STURGEON, N. S. MAGOSKI;
Dept. Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada

Abstract: Cation channels control excitability and are tightly regulated by a variety of modulators. A Ca^{2+}-dependent, voltage-gated, non-selective cation channel provides depolarizing drive to maintain the afterdischarge - a prolonged period of enhanced excitability in bag cell neurons required to initiate reproduction in *Aplysia californica*. Using single-channel recording, the Ca^{2+} activation of the cation channel and its response to modulators was investigated. Phosphatidylinositol-4,5-bisphosphate (PIP_2) breakdown is increased during the afterdischarge, yielding increased amounts of inositol triphosphate (IP_3) and diacylglycerol (DAG). We have previously reported that IP_3 dramatically right-shifts the Ca^{2+}-dependence of the cation channel (from EC_{50} 30μM to 20mM). We now find IP_3 competes with Ca^{2+} activation of the channel, reducing the P_o by 50% in the presence of high Ca^{2+}. Perfusing the same excised patch with high Ca^{2+} and no IP_3 did not reactivate the channel, suggesting IP_3 has sustained effects or binds tightly to the channel. Because PIP_2 and DAG modulate cation channels in general, the influence of these compounds on the *Aplysia* cation channel will be addressed, along with arachidonic acid, an important second messenger precursor. The *Aplysia* cation channel is transient receptor potential (TRP) channel-like. TRP channels play critical roles in a diverse group of physiological processes. Accordingly, the TRP channel inhibitors 9-phenanthrol (9PT), flufenamic acid (FFA), and SKF-96365 (SKF) all reduced the P_o of the cation channel in excised, inside-out patches. FFA, an inhibitor of many TRP channels, moderately reduced P_o by 45%, while 9PT, a specific inhibitor of TRPM4/5 channels, and SKF drastically decreased P_o by 90% and 80% respectively. In an effort to functionally compare the *Aplysia* cation channel to TRP channels, we have initiated cloning and expression of *Aplysia*-TRP-M and -C homologues in HEK293 cells. Characterizing the native *Aplysia* cation channel, and comparing its pharmacological properties with that of the *Aplysia*-TRP channels, will provide a molecular foothold towards determining the specific role for TRP channels in bag cell neurons. At present, we conclude that a TRP-like cation channel is tightly controlled by multiple modulators and its local lipid environment, providing a balance for initiating and maintaining the afterdischarge to regulate reproduction.

Disclosures: R.M. Sturgeon: None. **N.S. Magoski:** None.**Poster**

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.01/D42

Topic: B.03. G-Protein Linked Receptors

Title: An investigation into the binding site of M1 muscarinic acetylcholine receptor ligands

Authors: *A. J. MOGG, M. CRABTREE, L. M. BROAD;
Eli Lilly & Co. Ltd, Windlesham, United Kingdom

Abstract: Activation of the M1 muscarinic acetylcholine receptor (mAChR) is mediated either by binding at the orthosteric acetylcholine binding site or by interactions at allosteric sites on the M1 mAChR. Additionally some M1 ligands are reported to be bi-topic, with binding spanning both the orthosteric and allosteric sites.
The principle focus of this study was to investigate the binding mechanism of a range of muscarinic ligands including examples reported to be orthosteric (e.g. acetylcholine, Xanomeline, pilocarpine, oxotremorine-M) versus examples thought to be allosteric or bi-topic (e.g. BQCA, GSK-5).
The orthosteric radioligand ([^{3}H]N-methyl-scopolamine was used to compare the displacement profile of these ligands versus this prototypical orthosteric antagonist. Experiments were also conducted to assess if this profile of displacement was altered in the presence of BQCA, an M1 positive allosteric modulator. We also synthesised and characterised a novel [^{3}H]-radioligand thought to bind at an allosteric site on the M1 receptor to conduct further analysis of the binding of the ligands. In addition to competition binding assays with these radioligands, kinetic off-rates were also measured as an additional approach to deduce mechanism of binding.
The results confirm the allosteric mechanism of BQCA, but raise questions over the mechanism of binding of other agents previously reported to be either orthosteric or allosteric ligands. Additional studies will need to be conducted to help us confidently interpret these new findings.

Disclosures: A.J. Mogg: None. **M. Crabtree:** None. **L.M. Broad:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.02/E1

Topic: B.03. G-Protein Linked Receptors

Title: Back-translation of clinical data into preclinical models: De-risking strategy for M1-PAM

Authors: *K. DE WAEPENAERT[1], F. ROMBOUTS[2], L. LENAERTS[3], I. FONTEYN[4], T. SMETS[1], M. MAHIEU[5], H. HENDRICKX[5], D. SMETS[1], R. MOSTMANS[1], A. VANLOMMEL[1], S. JANSSENS[1], F. COOLS[1], A. MEGENS[5], E. CLESSENS[1], H. BORGHYS[1], M. SOMERS[1], G. VANHOOF[1], J. AERSSENS[1];
[1]C.R.E.A.Te Translational Sci., [2]Neurosci. Medicinal Chem., [3]C.R.E.A.Te Core scientific Technologies, [4]C.R.E.A.Te Mol. sciences, [5]Dept. Neurosci., Janssen Res. and Development, A Div. of Janssen Pharmaceutica NV, Beerse, Belgium

Abstract: Alzheimer's disease is a severe and chronic mental illness for which only symptomatic treatment is currently available. Marketed drugs (eg Donepezil, Aricept®) enhance the cholinergic system by increasing the endogenous acetylcholine levels through inhibition of acetylcholinesterase. This results in a modest cognition-enhancing effect combined with side effects including nausea, diarrhea, salivation, vomiting and dizziness related to the non-selective activation of all subtypes of the muscarinic and nicotinic receptors. Preclinical evidence suggest that the M1 muscarinic receptor subtype plays an important role in cognition enhancement while activation of muscarinic M2 and M3 receptors are involved in the development of side effects. Selective activation of M1 by pursuing allosteric modulation, e.g. ectopic agonists or positive allosteric modulators (PAM) could boost the cognitive performance without the reported side effects. Recently however, it was reported that treatment with a selective M1 ectopic agonist did cause salivation and vomiting as side effects in a clinical study at low exposure levels in plasma. Therefore, a de-risking strategy was outlined to clarify whether selective positive allosteric modulation of M1 is a viable way forward for cognition enhancement in Alzheimer disease by characterizing a set of tool compounds with known diverse affinities to the different muscarinic receptors: an acetylcholinesterase inhibitor (Donepezil, Aricept®), ectopic agonists (GSK1034702, JNJ49791300) and M1 PAMs (BQCA, PQCA, JNJ54784964, Merck WO201184368).
M1 PAMs showed a specific M1 profile in a recombinant selectivity panel, consisting of the 5 human muscarinic receptors. Target engagement was demonstrated by IP1 increase both in primary rat hippocampal neurons and in rodent hippocampal brain extracts upon treatment with all compounds.
Ectopic agonists did not evoke the side effects that were reported in the clinic in rat in vivo models of proconvulsant activity, locomotor actvity, diarrhea, miosis, hypothermia and salivation. In contrast, in dog, ectopic agonists induced diarrhea, salivation and vomiting indicating that the dog is more sensitive than rodents for peripheral muscarinic-induced side effects. However, side effects induced by M1 PAMs were observed in rodents as well as in dogs at minimal exposures expected to be needed for target engagement. In conclusion, using in vitro and in vivo translational models, we enabled a data-driven decision to stop the further development of M1 PAMs.

Disclosures: K. De Waepenaert: None. **F. Rombouts:** None. **L. Lenaerts:** None. **I. Fonteyn:** None. **T. Smets:** None. **M. Mahieu:** None. **H. Hendrickx:** None. **D. Smets:** None. **R. Mostmans:** None. **A. Vanlommel:** None. **S. Janssens:** None. **F. Cools:** None. **A. Megens:** None. **E. Clessens:** None. **H. Borghys:** None. **M. Somers:** None. **G. Vanhoof:** None. **J. Aerssens:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.03/E2

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant U54 MH084659

Vanderbilt University is a Specialized Chemistry Center within the MLPCN

Title: Discovery, synthesis, and pharmacological characterization of novel, highly selective m5 allosteric modulators

Authors: *P. R. GENTRY[1,2,3,4], M. KOKUBO[1,2,6], D. J. FOSTER[1,2,3,5], T. M. BRIDGES[1,2,3,5], C. M. NISWENDER[1,2,3,5], J. S. DANIELS[1,2,3,5], P. J. CONN[1,2,3,5], M. R. WOOD[1,2,3,4,5], C. W. LINDSLEY[1,2,3,4,5];
[2]Vanderbilt Ctr. for Neurosci. Drug Discovery, [3]Vanderbilt Specialized Chem. Ctr. for Probe Develop. (MLPCN), [4]Dept. of Chem., [5]Dept. of Pharmacol., [1]Vanderbilt Univ., Nashville, TN; [6]Ono Pharmaceut., Osaka, Japan

Abstract: The muscarinic acetylcholine receptors (mAChR) are a family of class A G protein-coupled receptors comprised of five subtypes (M_{1-5}) expressed throughout the central nervous system (CNS). These receptors, together with their endogenous agonist, acetylcholine (ACh), play a vital role in regulating a wide range of physiological functions. Recent advances in the discovery of highly subtype-specific ligands for M_1 and M_4 have enabled researchers to pharmacologically elucidate the discrete functions of these subtypes in the CNS. However, discovery of M_5-selective ligands has remained challenging due to the highly conserved nature of the orthosteric site across the mAChR subtypes. Phenotypic studies of M_5-knockout mice have suggested that activation of M_5 may be therapeutically relevant for the treatment of chronic cerebrovascular diseases, acute ischemic stroke, and Alzheimer's disease. Furthermore, M_5's localization in dopaminergic neurons of the midbrain suggests that modulation of M_5 signaling may provide novel therapies for the treatment of Parkinson's disease and addictive behavior. Here we use parallel synthesis and in vitro molecular pharmacology techniques to identify,

optimize, and pharmacologically characterize highly selective M_5 positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) for use as probes to elucidate the role of M_5 in the CNS. We recently reported the discovery of the first submicromolar, highly-selective M_5 PAM, ML326 (VU0467903; hM_5 EC_{50} = 409 nM), but its poor physiochemical profile limited its utility as a pharmacological probe. At this point, a high throughput screen for M_5-specific ligands was performed on the MLPCN screening deck. Gratifyingly, this effort revealed a novel M_5 PAM scaffold along with a scaffold functioning as an M_5 antagonist. The PAM scaffold, VU0472882 (hM_5 EC_{50} = ~19 μM), was subjected to a multi-dimensional, iterative parallel synthesis effort in which multiple regions of the molecule were manipulated and juxtaposed to optimize the structure-activity relationships. This effort yielded an M_5-preferring PAM with a 100-fold improvement in potency, VU0481443 (hM_5 EC_{50} = 190 nM). The antagonist scaffold, VU0352221 (hM_5 IC_{50} = 3.5 μM) was similarly optimized, yielding VU0483253 (hM_5 IC_{50} = 300 nM). Further radioligand binding experiments have revealed VU0483253 to be acting allosterically; thus, VU0483253 represents the first submicromolar, highly M_5-selective NAM. Moreover, VU0483253 shows a favorable rat pharmacokinetic profile with low clearance (CL_p ~4.4 mL/min/kg), long half-life ($t_{1/2}$ ~16 hr), and good CNS distribution (brain-plasma K_p ~3.3).

Disclosures: P.R. Gentry: None. **M. Kokubo:** A. Employment/Salary (full or part-time):; Ono Pharmaceutical. **D.J. Foster:** None. **T.M. Bridges:** None. **C.M. Niswender:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb. **J.S. Daniels:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb. **M.R. Wood:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb. **C.W. Lindsley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.04/E3

Topic: B.03. G-Protein Linked Receptors

Support: NIH NINDS1R01-NS065867

UDAL Emory 4042080053

Title: Characterization of Novel M4 PAM in Huntington's disease mouse model

Authors: *T. PANCANI, A. B. BOWMAN, T. J. BICHELL, T. M. BRIDGES, D. J. SCOTT, C. W. LINDSLEY, C. JONES, P. J. CONN, Z. XIANG;
Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ. Medcenter, Nashville, TN

Abstract:
Huntington disease (HD) is a neurodegenerative disease characterized by severe motor and behavioral alterations. The appearance of a hyperkinetic phenotype (chorea) marks the beginning of the motor symptomatic stage, is followed by akinesia and progressive conspicuous loss of striatal medium spiny neurons (MSNs). The causes responsible for this loss of striatal parenchyma are not understood; however, changes in signaling by glutamate and other neurotransmitters, largely precede the onset of the symptomatic stage of the disease, and might be responsible for behavioral alteration and striatal neuronal loss. Similar to humans, at an early age, HD mice show motor hyperactivity and stereotypic behavior, associated with increased striatal glutamate transmission, while late akinesia, is accompanied by striatal neuronal loss and decreased glutamatergic signaling. Data suggest that striatal cholinergic activity and function of muscarinic receptors (mAChRs) are compromised in HD, while clinical studies show that some cholinesterase inhibitors can provide significant symptomatic relief. M4 is one of the functionally predominant mAChR subtypes in the striatum. We now report that M4 activation inhibits evoked excitatory post-synaptic currents (eEPSCs), in MSNs by stimulation of cortico-striatal afferents in striatal slices. Furthermore, we found that eEPSC peak amplitude is increased in 50 days old YAC128 mice compared to WT, as shown previously. Accordingly, the amplitude of spontaneous EPSCs (sEPSCs) is increased in YAC128 mice. In these mice, an increase in glutamatergic transmission is accompanied by an increased M4-mediated inhibition of cortico-striatal eEPSCs, suggesting an increased M4 function at the cortico-striatal synapses and a decrease of striatal acetylcholine release previously described in HD. To investigate if pharmacological manipulation of M4-mAChRs would improve motor symptoms in early stage HD, we monitored the effects of a new M4 positive allosteric modulator, VU0467154, in open field, clasping and gait behavioral tests in 50 days old YAC128 mice. Our work supports the notion that M4 might represent a new therapeutic target for the treatment of HD.

Disclosures: T. Pancani: None. **A.B. Bowman:** None. **T.J. Bichell:** None. **T.M. Bridges:** None. **D.J. Scott:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca and Bristol-Myers Squibb. **C.W. Lindsley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca and Bristol-Myers Squibb. **C. Jones:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca and Bristol-Myers Squibb. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca and Bristol-Myers Squibb. **Z. Xiang:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.05/E4

Topic: B.03. G-Protein Linked Receptors

Support: MH95285

MH073676

NS71669

Title: M5-muscarinic receptors engender different physiological outcomes in SNc neurons depending on their subcellular location

Authors: *D. J. FOSTER, P. R. GENTRY, Z. XIANG, C. W. LINDSLEY, P. J. CONN; Vanderbilt Ctr. For Neurosci. Drug Discovery, Nashville, TN

Abstract: Dysregulation of dopamine release from midbrain dopamine neurons is thought to underlie numerous disorders including Parkinson's disease and Attention Deficit Hyperactivity Disorder (ADHD). These midbrain neurons receive autonomous cholinergic afferents to their soma in the midbrain and to their terminals in the striatum. The only member of the muscarinic receptor (mAChR) family detectible in midbrain dopaminergic neurons is the M5 subtype. Here we use a combination of pharmacological and genetic tools to elucidate the role of this receptor in regulating substantia pars compacta (SNc) neuron physiology. At the SNc soma, the presence of functional M5 receptors was verified using an M5-selective positive allosteric modulator (VU0238429). In wild type animals addition of the non-selective mAChR agonist oxotremorine (Oxo-M) induced both inward currents and Ca2+ mobilization in these neurons. Concentrations of Oxo-M that produced submaximal currents / Ca2+ mobilization could be potentiated by inclusion of VU0238429. Furthermore, these responses were absent in M5 knock-out mice, suggesting that M5 is the only mAChR subtype mediating these effects. In wild type animals Oxo-M induced changes in firing rate as monitored using perforated patch recordings. Surprisingly, VU0238429 did not potentiate the increase in firing rate observed with sub-maximal Oxo-M concentrations, suggesting that M5 may play a more modulatory role at this subcellular localization. In the striatum, addition of Oxo-M induced an inhibition in dopamine release as monitored using cyclic voltammetry. Furthermore, VU0238429 potentiated the inhibition observed with submaximal Oxo-M doses suggesting that M5 activation in the striatum inhibits dopamine release. These results suggest that activation of the same receptor type in the same neuron can lead to very different physiological outcomes. While activation of somatic M5

leads to inward currents, activation of M5 in the neuron terminals induces an inhibition in neurotransmitter release.

Disclosures: D.J. Foster: None. **Z. Xiang:** None. **C.W. Lindsley:** None. **P.J. Conn:** None. **P.R. Gentry:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.06/E5

Topic: B.03. G-Protein Linked Receptors

Support: NIH/NINDS 5R01NS065867

NIH/NINDS 5P50 NS071669

Title: Selective activation of cholinergic interneurons induces long-lasting enhancement of intrinsic excitability of striatal medium spiny neurons

Authors: X. LV, C. LINDSLEY, P. J. CONN, *Z. XIANG;
Vanderbilt Univ., Nashville, TN

Abstract: The striatum is the primary gateway to the basal ganglia and critically involved in motor behaviors, habit formation and cognition, and its function is regulated by multiple neuromodulator systems including cholinergic systems. Cholinergic regulation of striatal function is mediated by acetylcholine (ACh) released from tonically active, giant aspiny cholinergic interneurons (ChIs) that have widespread and rich axonal arborizations within the striatum and acting on nicotinic and muscarinic receptors. Disturbance of striatal cholinergic system has been implicated in numerous neurologic and neuropsychiatric disorders including in Parkinson's disease, Huntington's disease, dystonia, Tourette syndrome and Schizophrenia. Of five muscarinic acetylcholine receptor (mAChR) subtypes, the M1 is one of the most abundant mAChR subtypes in the striatum. This receptor is highly expressed in both striatonigral and striatopallidal projection medium spiny neurons (MSNs). In previous studies, we and others have shown that cholinergic activation excites MSNs and this action is mediated by M1 receptors through modulating multiple potassium channels. In the present study, we used electrophysiology techniques in conjunction with optogenetic and pharmacological tools to determine the long-term effects of endogenous ACh release on MSN intrinsic excitability. The experiments were performed in acute striatal slices prepared from ChAT-ChR2(H134R)-EYFP transgenetic mice in which ChR2-EYFP was selectively expressed in ChIs. Whole cell current

clamp recordings were made from MSNs. An increase in endogenous ACh release was triggered by selective activation of ChIs that express ChR2 using blue laser light stimulation. We found that a brief increase in ACh release results in a long-lasting increase in excitability of MSNs, which is associated with hyperpolarization of action potential threshold and leads to increase in probability of EPSP-action potential coupling. In the presence of M1 selective antagonist VU0255035, the optical stimulation is no longer able to induce persistent increase in MSN excitability. This endogenous ACh induced and M1 receptor dependent long-lasting change in MSN excitability could have significant impact on striatal processing and might provide a novel mechanism underlying cholinergic regulation of the motor behavior and cognitive function.

Disclosures: X. Lv: None. **C. Lindsley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca and Bristol-Myers Squibb. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca and Bristol-Myers Squibb. **Z. Xiang:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.07/E6

Topic: B.03. G-Protein Linked Receptors

Support: FAPESP 11/51600-2

INCTTOX

Title: Effects of an anti-muscarinic component isolated from Micrurus lemniscatus venom on intracellular signaling by inositol phosphate and learning and memory of rats

Authors: *M. L. SANDOVAL, SR[1], T. S. SATAKE[1], M. O. CASOTTI[1], E. O. FRARE[1], T. J. OLIVEIRA[1], R. M. PORTO[1], I. F. C. BATISTA[1], F. M. ABDALLA[1], G. F. XAVIER[2]; [1]Lab. of Pharmacol., Butantan Inst., São Paulo, Brazil; [2]Lab. of neuroscience and behavior, Inst. of Biosciences/University of São Paulo, São Paulo, Brazil

Abstract: Elapid venoms usually exhibit muscarinic cholinergic components. The protein MTMlβ exhibits a N-terminal sequence determined by Edman degradation (NLYQFKNMIQCTNTRSCLDFADYGCYCGRGGSCT) and displays high similarity to proteins from Elapidae venoms already described, including a muscarinic inhibitor from *N. sputatrix*. This study investigated the effects of MTMlβ isolated from *M. lemniscatus* venom, on (1) the displacement of the hippocampal (Hpc) muscarinic antagonist [^{3}H]QNB, (2) the levels of

inositol tri-phosphate, and (3) performance of rats in the Morris' water maze. In experiments of saturation, Hpc membranes were incubated with [^{3}H]QNB (0,05-8,0 nM) both in the absence and presence of atropine (1 M) (30°C/1h). Scatchard analysis of specific binding yielded a dissociation constant (K_D)=0.88 ± 0,13 nM and binding capacity (B_{max}) = 1459.40 ± 235.26 fmol/mg of protein (n=5). Hpc membranes were also incubated with [^{3}H]QNB, both in the absence and presence of increasing concentrations of the MTMlβ and atropine (control) (30°C/1h). MTMlβ and atropine revealed one high affinity muscarinic binding site (respectively, pK_i = 7.38 0.15, n=4 and pK_i = 8.96 ± 0.08, n=4) to [^{3}H]QNB in the hippocampus. The MTMlβ (10^{-7} M) reduced the accumulation of intracellular [^{3}H] - inositol phosphates content in the rat hippocampus as induced by carbachol (10^{-5} M). Rats subjected to training in a working memory version of the Morris' water maze task, with the platform in a different location every day and 4 trials per day, received, on the 8th day, a Hpc injection (0.25µg/µL) of either MTMlβ or phosphate buffer (Controls) twenty minutes before training. Their performance did not differ significantly from that exhibited by the Control subjects. On the 9th day, however, when tested in the same task without any microinfusion, the subjects previously injected with MTMlβ exhibited significantly longer latencies and path lengths as compared to the Control subjects ($P < 0.0004$), and significant longer times spent within the day before critial quadrant ($P < 0.0083$). Interestingly, there were no significant main Trial and Trial x Group interaction effects ($P > 0.05$). These results indicate that the disruption of performance observed on the 9th day may be related to a better retrieval of the information about the platform location acquired on the previous day, during the MTMlβ effect. Hpc histology did not reveal any neuronal injury. In conclusion, the MTMlβ exhibits affinity for mAChRs and reduces the total inositol phosphates, revealing a profile of muscarinic antagonist in the rat hippocampus. The memory effects induced by MTMlβ intra-cerebral infusion deserve additional investigation.

Disclosures: M.L. Sandoval: None. **T.S. Satake:** None. **M.O. Casotti:** None. **E.O. Frare:** None. **T.J. Oliveira:** None. **R.M. Porto:** None. **I.F.C. Batista:** None. **F.M. Abdalla:** None. **G.F. Xavier:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.08/E7

Topic: B.03. G-Protein Linked Receptors

Support: CIHR MOP 89825 (EKL)

Ontario Early Researcher Award (EKL)

Canada Research Chairs (EKL)

OMHF Studentship (EP)

Intramural funds from TIFR (VAV)

Title: Early stress prevents the potentiation of muscarinic excitation by calcium release in adult prefrontal cortex

Authors: *E. PROULX[1], D. SURI[2], S. P. HEXIMER[1,3], V. A. VAIDYA[2], E. K. LAMBE[1,4]; [1]Physiol, Univ. Toronto, Toronto, ON, Canada; [2]Tata Inst. for Fundamental Res., Mumbai, India; [3]Heart and Stroke/Richard Lewar Ctr. of Excellence in Cardiovasc. Res., Toronto, ON, Canada; [4]Obstetrics and Gynaecology, Univ. of Toronto, Toronto, ON, Canada

Abstract: The experience of early stress contributes to the etiology of several psychiatric disorders and leads to lasting cognitive deficits, particularly in executive function. The modulation of the prefrontal cortex by muscarinic M1 acetylcholine (ACh) receptors is essential to these functions. These Gαq-protein coupled receptors trigger the release of calcium (Ca2+) ions from internal stores in addition to eliciting prolonged neuronal excitation. We used multiphoton Ca2+ imaging simultaneously with whole-cell electrophysiological recordings to demonstrate that ACh-induced Ca^{2+} release potentiates ACh-elicited excitatory currents in pyramidal neurons of prefrontal brain slice. The enhancement was sensitive to manipulations of intracellular Ca2+ as well as to interference with electrogenic Na+/Ca2+ exchange. This phenomenon was found to emerge in young adulthood, at a time when executive function typically reaches maturity. However, such developmental consolidation of muscarinic ACh signaling was abolished subsequent to the early stress of repeated maternal separation. Under these conditions, the adolescent phenotype was retained and developmental disruptions in the expression of multiple relevant genes were observed. Taken together, this work illustrates cellular mechanisms that may allow early stress to disrupt cognitive performance on tasks requiring mature executive function.

Disclosures: E. Proulx: None. **D. Suri:** None. **S.P. Heximer:** None. **V.A. Vaidya:** None. **E.K. Lambe:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.09/E8

Topic: B.03. G-Protein Linked Receptors

Support: Swiss National Science Foundation (3100A0-117816)

Title: Phosphorylation of GABAB receptors controls the activity of its auxiliary subunits

Authors: L. ADELFINGER, R. TURECEK, K. IVANKOVA, *M. GASSMANN, B. BETTLER;
Univ. of Basel, Basel, Switzerland

Abstract: GABAB receptors are the G-protein coupled receptors (GPCRs) for γ-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter in the central nervous system. GABAB receptors are examples of GPCRs that are composed of principal and auxiliary subunits. The principal subunits have a typical seven-transmembrane domain topology and form fully functional heteromeric GABAB(1a,2) and GABAB(1b,2) receptors. These receptors bind the auxiliary K-channel tetramerization domain-containing proteins KCTD8, 12, 12b and 16 that influence receptor signaling (Schwenk et al., 2010, Nature 465:231-5). In particular, binding of the auxiliary subunit KCTD12 increases fast desensitization of receptor-mediated Kir3 currents. The mechanism underlying the desensitizing effect of KCTD12 on GABAB-mediated receptor responses are unknown. In principle, KCTD12 could influence the GABAB(1,2) core receptor, the G protein activation-deactivation cycle and/or the downstream Kir3 channel.
We now report that KCTD12-mediated desensitization of GABAB receptor responses is alleviated by protein kinase A activity. We have mapped a phosphorylation site in the receptor that mediates the effects of protein kinase A on KCTD12-mediated desensitization. We will provide an update on progress made in identifying the underlying mechanism.

Disclosures: L. Adelfinger: None. **R. Turecek:** None. **K. Ivankova:** None. **M. Gassmann:** None. **B. Bettler:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.10/E9

Topic: B.03. G-Protein Linked Receptors

Support: NHMRC program grant 400121

NMHRC fellowship 1005050

ARC Future Fellowship FT0990628

Title: KCTD modulation of GABA(B) receptor function

Authors: M. Y. S. LI[1], C. J. MILLIGAN[1], H. WANG[2], *C. A. REID[1], S. C. HOPKINS[2], S. PETROU[1];
[1]Ion channel and disease group, Florey Neurosci. Inst., Melbourne, Australia; [2]Sunovion Pharmaceuticals Inc., Marlborough, MA

Abstract: Aim: KCTD12 and KCTD16 were identified as GABA(B) receptor auxiliary subunits, yet their functional significance has yet to be clearly elucidated. In order to better understand the functional role of these subunits, we characterized the in vitro impact of human KCTD12 and KCTD16 on GABA¬(B) receptor kinetics and pharmacology and characterized in vivo seizure susceptibilities using the KCTD12 knockout mice. Methods: For in vitro analysis we used a Xenopus laevis oocyte based automated two-electrode voltage clamp assay. GABA(B) receptor activated GIRK current was used to characterize the effects of the GABA(B) receptor agonist baclofen or the positive allosteric modulator CGP7930 on receptor kinetics in the absence and presence of KCTD subunits. In vivo studies compared the seizure susceptibility of the KCTD12 knockout mice with their wild type littermates to two seizure paradigms: thermogenic stress and PTZ induced seizures. For the thermogenic stress study, P14-16 mice were subjected to constant 42 Celsius degrees and the time to febrile seizure was recorded. For the PTZ induced seizure study, P40 mice were injected with PTZ (100mg/kg) and the latency to hind leg extension was measured. Results: Co-expression of KCTD12 with GABA(B) receptor accelerated the kinetics of response to agonist application (20-80% rise time from 2.8s to 1.3s) and the kinetics of subsequent receptor desensitization (increased from 3.9% to 54.7%, compared to GABA(B) receptor expression alone, n>7 oocytes). In contrast, KCTD16 co-expression did not significantly alter GABA(B) receptor kinetics. Analysis of baclofen dose-response curves showed that neither KCTD12 nor KCTD16 co-expression altered the EC50 or Hill slope (n>10 oocytes at each agonist concentration). However, the potentiating effect of CGP7930 on an EC20 concentration of GABA was enhanced in the presence of either KCTD12 or KCTD16 (from 11.5% to 20.3% and 24.4% respectively, n>33 oocytes). In the in vivo seizure studies, the sensitivity of thermogenic stress induced seizures was similar between KCTD knockout mice and wild type (n=7). However, a significant protection from PTZ induced seizure was observed in the KCTD12 knockout mice (survival rate from 33.3% to 81.8%, n=9-11). Conclusion: KCTD12 altered the kinetics of GABA(B) receptor, allosteric modulation and seizure susceptibility, presumably reflecting altered neuronal excitability. This raises the possibility of selective targeting of interactions between KCTDs and GABA(B) receptors.

Disclosures: M.Y.S. Li: None. **C.J. Milligan:** None. **H. Wang:** None. **C.A. Reid:** None. **S.C. Hopkins:** A. Employment/Salary (full or part-time):; full-time employee of Sunovion Pharmaceuticals Inc. **S. Petrou:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.11/E10

Topic: B.03. G-Protein Linked Receptors

Support: American Heart Association Scientist Development Grant

Title: Enhanced postsynaptic $GABA_B$ receptor activity regulates excitatory neuronal architecture

Authors: *M. TERUNUMA[1], R. REVILLA-SANCHEZ[1], M. N. PANGALOS[2], S. J. MOSS[1,3]; [1]Dept Neurosci, Tufts Univ., BOSTON, MA; [2]Innovative Medicines, AstraZeneca, Alderley Park, United Kingdom; [3]Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: $GABA_B$ receptors are heterodimeric G protein coupled receptors composed of R1 and R2 subunits that mediate slow synaptic inhibition in the brain. Postsynaptic $GABA_B$ receptors which negatively regulate cAMP levels are predominantly found on dendritic spines adjacent to excitatory synapses and regulate neuronal activity. We have previously reported that $GABA_B$ receptors are intimately associated with protein phosphatase 2A and directly dephosphorylate S783 in the R2 subunit to enhance $GABA_B$ receptor endocytosis (Terunuma et al., PNAS, 2010). To examine the significance of postsynaptic $GABA_B$ receptors as a long-term determinant of neuronal activity, we have generated a $GABA_B$R2 subunit Serine to Alanine mutant knock-in mouse (S783A) to prevent S783 dephosphorylation. S783A mice expressed stable $GABA_B$ receptors on the plasma membrane and the basal PKA activity was significantly reduced. Reduced PKA activity led to decreased PKA-mediated phosphorylation of cAMP-response element binding-protein (CREB) and immediate early gene Arc/Arg3.1 expression, two proteins necessary for spatial memory consolidation. In addition, we found an elevated surface AMPA receptor expression and increased number of excitatory synapses, which paralleled with reduced Arc/Arg3.1 expression. Our study demonstrated a novel role of postsynaptic $GABA_B$ receptors regulating excitatory synaptic architecture.

Disclosures: M. Terunuma: None. **R. Revilla-Sanchez:** None. **S.J. Moss:** F. Consulting Fees (e.g., advisory boards); SAGE Therapeutics, AstraZeneca. **M.N. Pangalos:** A. Employment/Salary (full or part-time):; AstraZeneca.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.12/E11

Topic: B.03. G-Protein Linked Receptors

Title: The effects of baclofen and phaclofen on performance in the Morris water maze

Authors: *C. F. HEANEY, M. M. BOLTON, A. S. MURTISHAW, J. W. KINNEY;
Dept. of Psychology, Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: The primary inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), has been implicated in regulating multiple neural processes including oscillations and long-term potentiation (LTP), as well as complex behavior such as learning and memory. Investigations have established that GABA is involved with neurons being entrained into oscillatory firing patterns, and these firing patterns have been shown to be beneficial for LTP and learning and memory. The metabotropic GABAB receptor has also been demonstrated to affect oscillations and LTP, however the role of this receptor in learning and memory tasks has not been as fully characterized as other GABA receptors. Limited data exist on the behavioral effects of altering GABAB receptor function in learning and memory tasks, and the results are varied. Utilizing male Sprague-Dawley rats, we tested the effects of the GABAB agonist, baclofen, and the GABAB antagonist, phaclofen, in the Morris water maze. Our results indicate that both ligands induced a change in learning and memory behavior in this task. We also analyzed hippocampal tissue for alterations to numerous protein markers and have found changes that may be related to the behavioral differences. These data indicate that alterations to GABAB receptor function may induce changes in learning and memory, and suggest a more prominent role for GABAB-mediated signaling in complex behavior.

Disclosures: C.F. Heaney: None. **M.M. Bolton:** None. **A.S. Murtishaw:** None. **J.W. Kinney:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.13/E12

Topic: B.03. G-Protein Linked Receptors

Support: NRF 2013-005232

Title: Natural substance neuroprotect against ethanol-induced neuronal apoptosis via $GABA_B$ receptors intracellular signaling in prenatal rat hippocampal neurons

Authors: *M.-O. KIM, S. ALI SHAH, I. ULLAH, T. KIM, G. YOON, H. LEE;
., Dept. of Biol., Gyeongsang Natl. Univ., GAZA 900, Jinju, Korea, Republic of

Abstract: Here we investigated the possible involvement of gamma-aminobutyric acid B1 receptor ($GABA_{B1}R$) in mediating the protective effects of black soybean anthocyanins against ethanol-induced apoptosis in prenatal hippocampal neurons because GABARs are known to play an important role in the development of central nervous system. Treatments were performed on primary cultures of prenatal rat hippocampal neurons transfected with or without $GABA_{B1}R$ small interfering RNA (siRNA). The results showed that when ethanol treatment was followed by anthocyanins treatment, cellular levels of pro-apoptotic proteins such as Bax, activated caspase-3 and cleaved poly (ADP-ribose) polymerase 1 (PARP-1) were decreased and the cellular level of the anti-apoptotic protein Bcl-2 was increased compared to treatment with ethanol alone. Furthermore, the effects of ethanol on cellular levels of $GABA_{B1}R$ and its downstream signaling molecules such as protein kinase A, calcium/calmodulin-dependent protein kinase II (CaMKII) and phosphorylated cAMP response element binding protein (p-CREB) were diminished or reversed by anthocyanins treatment. The ability of anthocyanins to reverse the effects of ethanol on cellular levels of Bax, Bcl-2, active caspase-3, cleaved PARP-1, $GABA_{B1}R$ and CaMKII were abrogated in cells transfected with $GABA_{B1}R$ siRNA. In a $GABA_{B1}R$-dependent manner, anthocyanins also inhibited the ability of ethanol to elevate intracellular free Ca^{2+} level and increase the proportion of cells with low mitochondrial membrane potential in the population. Cell apoptosis assay and morphological studies also confirmed the neuroprotective effect of anthocyanins against ethanol via $GABA_{B1}R$. Our data suggest that $GABA_{B1}R$ plays an important role in the neuroprotective effects of anthocyanins against ethanol (This work supported by NRF 2013-005232).

Disclosures: M. Kim: None. **S. ali shah:** None. **I. Ullah:** None. **T. Kim:** None. **G. Yoon:** None. **H. Lee:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.14/E13

Topic: B.03. G-Protein Linked Receptors

Support: NSERC discovery grant

Title: GABA(B) receptors in the nucleus accumbens modulate dopamine release in a frequency dependent manner

Authors: *K. A. PITMAN[1], S. L. BORGLAND[2];
[1]UBC, Vancouver, BC, Canada; [2]Univ. of Calgary, Calgary, AB, Canada

Abstract: Behavioural studies have suggested that agonists of the γ-aminobutyric acid receptor type B (GABA(B)R) can decrease drug seeking. Consequently, GABA(B) agonists, such as R-baclofen, have been investigated for potential usage as anti-addictive compounds. Paradoxically, γ-hydroxybutyrate (GHB), a weak GABA(B) agonist, is abused recreationally. Ventral tegmental area (VTA) dopamine (DA) neurons and their projection to the nucleus accumbens (NAc) are part of a critical circuit mediating drug seeking behaviour. DA neurons fire at low frequencies but can fire in high frequency bursts and deliver higher terminal dopamine concentrations ([DA]) when animals are presented with reward-predicting stimuli. GABA(B)Rs are located in the NAc, however, it is unknown how activation of NAc GABA(B)Rs can modulate [DA]. Using fast scan cyclic voltammetry in brain slices containing the NAc core from adult male C57 Bl/6 mice, we found R-baclofen dose-dependently decreased DA release electrically evoked by a single pulse with an IC50 of 1.3 µM. The inhibition induced by R-baclofen (100 µM) was blocked by a GABA(B) antagonist (CGP52432, 1 µM). 10 mM GHB also decreased [DA]. Interestingly, R-baclofen-mediated or GHB-mediated inhibition of NAc [DA] was inversely proportional to stimulation frequency. Evoking dopamine (1 pulse or 5p 40 Hz stimulation) did not increase endogenous GABA effects at GABA(B)Rs in the NAc because CGP52432 alone had no effect on [DA]. Taken together, these data suggest that GABA(B) agonists acting at GABA(B)Rs in the NAc decrease [DA] in the NAc. The efficacy of inhibition of [DA] at burst-like frequencies by R-baclofen was greater than GHB suggesting a possible additional mechanism why, unlike GHB, R-baclofen may reduce craving.

Disclosures: K.A. Pitman: None. **S.L. Borgland:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.15/E14

Topic: B.03. G-Protein Linked Receptors

Title: Patch-clamp analysis of anti-spasticity effect by baclofen in spinal ventral horn neurons

Authors: T. ABE[1], *T. NAKATSUKA[2], W. TANIGUCHI[2], N. MINE[1], N. TAKIGUCHI[1], N. MIYAZAKI[1], M. YAMANAKA[1], M. YOSHIDA[1];
[1]Orthopaedic Surgery, Wakayama Med. Univ., Wakayama, Japan; [2]Kansai Univ. of Hlth. Sci., Osaka, Japan

Abstract: Gamma-aminobutyric acid (GABA) is one of the important inhibitory neurotransmitters in the central nervous system. GABA acts on GABA receptors classified into metabotropic G-protein-coupled $GABA_B$ receptor and ionotropic $GABA_A$ receptor. Severe spasticity caused by multiple sclerosis and spinal cord trauma cannot be adequately treated with oral antispastic medications. For the patients suffering from such spasticity, Baclofen, a $GABA_B$ receptor agonist is administered spinally via an implanted drug delivery device to treat spasticity and very effective. In order to determine the cellular mechanisms of $GABA_B$ receptor-mediated modulation of spinal transmission, we examined actions of baclofen on excitatory synaptic transmission in the ventral horn neurons using whole-cell patch-clamp recordings from spinal cord slices. In voltage-clamp mode (V_H = -70mV), the application of baclofen induced an outward current. Baclofen-induced outward current was observed in the presence of TTX or a non-NMDA receptor antagonist, CNQX. The baclofen-induced outward current was blocked by the addition of Cs^+ (K^+ channel blocker) or GDP-β-S (G protein activation inhibitor) in the pipette solution. The baclofen-induced outward current was mimicked by a $GABA_B$ receptor antagonist, CGP35348. These results indicate that baclofen mainly acts on postsynaptic motor neurons to induce an outward current via G-protein-mediated activation of K^+ channels through $GABA_B$ receptors. This may be a possible mechanism for anti-spasticity effect.

Disclosures: T. Abe: None. **T. Nakatsuka:** None. **W. Taniguchi:** None. **N. Mine:** None. **N. Takiguchi:** None. **N. Miyazaki:** None. **M. Yamanaka:** None. **M. Yoshida:** None.

Poster

516. **Muscarinic Acetylcholine Receptors and GABAB Receptors**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 516.16/E15

Topic: B.03. G-Protein Linked Receptors

Support: donation by Addex Therapeutics

Title: ADX71441 treatment in a rat model for Charcot-Marie-Tooth disease type 1A downregulates the Pmp22 gene overexpression and reduces number of hypomyelinated axons

Authors: *M. W. SEREDA[1], M. RUDOLPH[2], R. FLEDRICH[2], R. STASSART[2], S. M. POLI[3], H. HADDOUK[4], T. PRUKOP[2];
[2]Dept. of Neurogenetics, [1]Max-Planck Inst. Exp. Med., Goettingen, Germany; [3]Translational Sci., [4]Toxicology, Addex Therapeut., Plan Les Ouates, Switzerland

Abstract: Charcot-Marie-Tooth disease is the most common inherited neuropathy and a duplication of the peripheral myelin protein 22 gene on Chromosome 17 causes the most frequent subform Charcot-Marie-Tooth 1A. Patients develop a slowly progressive dysmyelinating peripheral neuropathy and distally pronounced muscle atrophy. The amount of axonal loss determines disease severity (Fledrich et al., 2012). Pmp22 gene and protein expression is downregulated in-vitro in Schwann Cells by GABAB receptor activation mediated by Baclofen (Magnaghi et al. 2004; Melcangi et al. 2005). Furthermore, knock-out mice for GABAB receptor show an upregulation of Pmp22 gene expression and interestingly thinner myelin sheaths reflecting CMT-like pathology (Magnaghi et al. 2008).
OBJECTIVE: To test if the GabaB positive allosteric modulator ADX71441, lowers Pmp22 gene expression and ameliorates the phenotype in a Pmp22 transgenic rat model ("CMT rats") for Charcot-Marie-Tooth disease type 1A.
METHODS: Following two initial short term pilot studies (5days treatment) conditions were set for the following therapy. Long-term therapy study in CMT rats (n=14) and wildtype rats (n=12) consisted of treatment with ADX71441 at 6mg/kg s.c. every second day for 5 weeks, then 3mg/kg s.c. daily for 4 weeks or vehicle (sesame oil, 1 mL/kg). At the start of the treatment the CMT and the wildtype rats were 4 week old. At the end of the 9 week treatment, Pmp22 gene expression, grip strength, electrophysiology, and axonal counting were measured as well as ADX71441 drug level in serum.
RESULTS:
CMT rats showed overexpression of the Pmp22 gene, an axonal loss and reduced grip strength reflecting typical CMT1A features. 9 weeks ADX71441 therapy in CMT rats downregulated the 1.6-fold Pmp22 gene overexpression, reduced the number of hypomyelinated axons and increased compound muscle action potentials in peripheral nerves. ADX71441 therapy in wildtype rats did not affect physiological Pmp22 gene expression, axonal number and electrophysiological parameters. However, ADX71441 in wildtype rats caused a reduction in rat grip strength. Since there is not a comparable loss of grip strength between CMT treated and untreated, the data suggests some benefit by ADX71441 on underlying pathophysiology
CONCLUSION: ADX71441 could lower toxic PMP22 overexpression and potentially delay the progression of the disease and therefore offer a unique therapeutic opportunity for CMT1A patients

Disclosures: M.W. Sereda: None. **M. Rudolph:** None. **R. Fledrich:** None. **R. Stassart:** None. **S.M. Poli:** A. Employment/Salary (full or part-time):; Addex Therapeutics. **H. Haddouk:** A. Employment/Salary (full or part-time):; Addex therapeutics. **T. Prukop:** None.**Poster**

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.01/E16

Topic: B.07. Synaptic Transmission

Title: Dynamic tuning of single neuron encoding through modulation of firing class and type of short-term plasticity

Authors: A. MOHAN[1], *C. STRICKER[2];
[1]Eccles Inst. of Neurosci., The Australian Natl. Univ., Canberra, Australia; [2]Dept. Neurosci, ANUMS / JCSMR, ANU, Canberra, Australia

Abstract: Synapses in neocortex show either release-dependent depression with a constant recovery rate (type 1) or release-independent depression with frequency-dependent recovery[1] (type 2). The firing characteristics can be classified as either class 1 (regular-firing) or class 2 (spike-frequency adapting)[2]. Based on a deterministic synapse model, we have shown that matching of type and class endows small networks with particular coding properties[3]. Here we focus on 2 aspects: Firstly, using electrophysiological recordings, we explore if firing class is under neuromodulatory control. Secondly, we study the encoding characteristics of all combinations between synapse type and firing class using a stochastic synapse model with one determining firing class.

Standard whole-cell recordings in layer IV of rat barrel cortex (14-18 d) were obtained. Firing was evoked by applying currents of 0.2 – 0.4 nA for 1.5 s every 30 s in the presence of either 10 µM noradrenaline (NA) or 20 µM 2-APB to block IP_3 receptors. Phase reset curves were measured using the direct method[4]. Bath-applied NA converted the firing with class 2 characteristics to that of class 1 (7 cells out of 9). In contrast, 2-APB converted class 1 firing to that of class 2 (6 cells out of 7). This suggests that NA can convert class 1 into class 2 firing and *vice versa* when IP_3 receptors are blocked.

Neurons were modeled with adaptive exponential Integrate-and-fire model and a previously developed stochastic model was used for the synapse. Stimulus typically consisted of 100 synapses relaying 1000 stimuli that were Gaussian-distributed in time. Timing of the first action potential was measured and averaged over 5000 trials. Based on reliability of the response and the standard deviation of the response time, we found that type 1 – class 2 acted as coincident while all other combinations as integrators.

Our results suggest that both pre- and postsynaptic dynamics shape single neuron encoding. Both dynamics are shaped by neuromodulators. We have previously shown that NA is preferential towards type 2 dynamics and now show that it also converts class 2 into 1 firing. This suggests that, for adrenergic modulation, both type 1-class 2 and class 2-type 1 configurations might be preferred configurations and that the dynamics are shifted *in tandem*, converting a coincidence detector into an integrator.

References:

[1] Fuhrmann G *et al. J Physiol* 557: 415–438, 2004.
[2] Hodgkin A *J Physiol* 107: 165-181, 1948

[3] Mohan A *et al*. *Front Comput Neurosci* 7: 41, 2013.
[4] Galán RF *et al. Phys Rev Lett* 94: 158101, 2005.

Disclosures: A. Mohan: None. **C. Stricker:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.02/E17

Topic: B.07. Synaptic Transmission

Support: NIH Training Grant NS007381

NIH Grant NS040056

Title: Activation of extrasynaptic NMDARs at individual PF - MLI synapses in cerebellum

Authors: *B. NAHIR, C. E. JAHR;
Vollum Inst., OHSU, Portland, OR

Abstract: NMDA receptors (NMDARs) expressed by cerebellar molecular layer interneurons (MLIs) are not activated by single exocytotic events but can respond to glutamate spillover following coactivation of adjacent parallel fibers (PFs), indicating that NMDARs are perisynaptic. Several types of synaptic plasticity rely on these receptors but whether they are activated at isolated synapses is not known. Using a combination of electrophysiological and optical recording techniques in acute slices of rat cerebellum, along with modeling, we find that repetitive activation of single PF-MLI synapses can activate NMDARs in MLIs. High frequency stimulation, multivesicular release (MVR), or asynchronous release can each activate NMDARs. Frequency facilitation was found at all PF-MLI synapses but, while some showed robust MVR with increased release probability (Pr), most were limited to univesicular release (UVR). Together, these results reveal a functional diversity of PF synapses, which use different mechanisms to activate NMDARs.

Disclosures: B. Nahir: None. **C.E. Jahr:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.03/E18

Topic: B.07. Synaptic Transmission

Title: Selective synaptic inhibition accompanying dendritic spine activity

Authors: *N. TAKAHASHI[1], N. MATSUKI[2], Y. IKEGAYA[2];
[1]NeuroCure, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Grad. Sch. of Pharmaceut. Sci., Univ. of Tokyo, Tokyo, Japan

Abstract: Dynamic balance of synaptic excitation and inhibition (E/I) shapes the membrane fluctuations of cortical neurons and determines the patterns of neuronal outputs. Although recent studies revealed the E/I balance during spontaneous or evoked activities at cellular level, the E/I balance at the subcellular level, i.e., individual synapses, remains poorly understood. Combined large-scale spine imaging with somatic voltage-clamp recording, we simultaneously recorded synaptic excitation at individual spines over dendrites and inhibitory postsynaptic currents at the soma. We found that, at 22% of active spines, synaptic events were accompanied by transient somatic inhibition within 100 ms, whereas at the other spines, activity occurred without apparent somatic inhibition. Compared to synapses without inhibition, inhibition-coupled spines had larger spine heads and showed greater amplitudes in calcium transients by synaptic activation, potentially contributing to strong membrane excitation. Our results designates a dynamic modulation of somatic inhibition for synaptic excitation at individual spines, suggesting a novel E/I balancing mechanism that gates specific synaptic pathways.

Disclosures: N. Takahashi: None. **N. Matsuki:** None. **Y. Ikegaya:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.04/E19

Topic: B.07. Synaptic Transmission

Support: European Research Council starting grant

Helmholtz Society

Deutsche Forschungs Gemeinschaft Exc 257 NeuroCure

Deutsche Forschungs Gemeinschaft "Barrel Cortex Function" SNF-DFG FOR 1341

Title: *In vivo* monosynaptic transmission between layer 2 GABA-ergic interneurons in mouse forepaw primary somatosensory cortex

Authors: *A. L. DORRN[1,2], J. F. A. POULET[1,2];
[1]Max-Delbrueck-Center For Mol. Med., Berlin, Germany; [2]NeuroCure - Neurosci. Res. Center, Charité-Universitaetsmedizin, Berlin, Germany

Abstract: In vivo measurements of monosynaptic transmission are necessary to characterize synaptic mechanisms underlying sensorimotor integration. Superficial layer cortical GABA-ergic interneurons form synaptic connections with both local excitatory and other GABA-ergic interneurons. Here we used double and triple two-photon targeted whole-cell recordings to measure GABA-ergic synaptic transmission between neighbouring layer 2 inhibitory interneurons in vivo in the urethane aneasthetised GAD67-GFP mouse forepaw primary somatosensory cortex. In a first step, we made spike-triggered averages from the spontaneous action potentials in putative pre- and postsynaptic neurons. This showed depolarizing peaks in nearby GABA-ergic interneurons during a spike suggesting strong common synaptic input to neighbouring interneurons thus making identification of monosynaptic connectivity between GABA-ergic interneurons using this method problematic. We therefore went on to trigger spikes with brief current injections across different brain states. This approach allowed us to investigate basic properties of inhibitory monosynaptic connections. It also revealed a strong impact of brain state on GABA-ergic synaptic transmission in vivo.

Disclosures: A.L. Dorrn: None. **J.F.A. Poulet:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.05/E20

Topic: B.07. Synaptic Transmission

Support: NIH Grant 5 T32 MH 67631-7

NIH Grant MH 084874

Title: Temporally and spatially distributed NMDA receptor-dependent synaptic input onto spinal ventral horn neuron dendrites during behavior

Authors: *M. H. ALPERT, S. ALFORD;
Biol. Sci., Univ. of Illinois, Chicago, CHICAGO, IL

Abstract: Vertebrate spinal ventral horn neurons possess elaborate dendritic arbors that transform both descending commands from the brainstem and those from local circuit activity into controlled movement. Glutamatergic and glycinergic synapses dispersed along dendrites actively shape neural output. However, little is known about how dendrites actively integrate spatially and temporally distinct synaptic inputs within intact neuronal networks during behavior. Thus, a fundamental question is: do physiological patterns of synaptic input reflect the output of individual neurons and how do these patterns impact behavior? To investigate this, we took advantage of the nearly intact, isolated lamprey CNS preparation. The brainstem locomotor control center can be stimulated to produce fictive swimming while the activity of individual neurons of the spinal ventral horn can be recorded using both imaging and electrophysiological modalities. During locomotion, spinal neurons receive phasic glutamatergic and glycinergic input, generating oscillations in membrane potential and intracellular Ca^{2+}. To examine both the electrical output of neurons and locations of NMDA-receptor dependent synaptic input, we combined sharp electrode electrophysiology with high-speed Ca^{2+} imaging. We demonstrate that spinal neurons show robust, phasic Ca^{2+} signals that are temporally and spatially variable throughout the dendritic tree. While these responses correspond to cellular activation and network swimming behavior, repetition of stereotypical activity either as patterns or as localized events fails to occur from cycle to cycle during locomotion. Discrete hotspots emerge, differing in both amplitude and temporal sequence, even amongst neighboring locations along a single dendrite. These signals exist subthreshold to spiking activity, but show enhancement during spikes, which can synchronize Ca^{2+} signals along and between dendritic branches. Ca^{2+} activity is abolished throughout the dendrites following local pressure-ejection of either the NMDA receptor antagonist, AP5, or the mGluR5 receptor antagonist, MPEP, while network activity persists. UV photo-uncaging of AP5 locally abolishes Ca^{2+} signals in discrete dendritic regions while sparing others. Thus, we conclude that dendrites of spinal ventral horn neurons receive locally distributed, NMDA receptor-dependent synaptic input during rhythmic network activity. This demonstrates that dendrites act as distributed computational devices in which the sum of dendritic input is powerfully transformed from a barrage of discrete and variable synaptic events into unified cellular output during behavior.

Disclosures: M.H. Alpert: None. **S. Alford:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.06/E21

Topic: B.07. Synaptic Transmission

Support: NIH K99 NS078118-02

NIH grant ns034774

Title: Evidence of a functional gabaergic inhibition between reticular thalamic neurons revealed by an optogenetic approach in the mouse

Authors: *J. PAZ, J. R. HUGUENARD;
Neurol. and Neurolog. Science, Stanford, Stanford Univ., STANFORD, CA

Abstract: The reticular thalamic nucleus (nRT) is involved in perception, sensation, attention, consciousness and in generation of sleep spindle oscillations in thalamocotical networks. Inhibition between nRT neurons (i.e., intra-RT inhibition) is proposed to be critical for normal functioning of the thalamus by desynchronizing nRT neuron activity through a burst-shunting mechanism (Sohal and Huguenard, 2003) and a disruption of intra-nRT inhibition has been proposed to lead to abnormal thalamocortical oscillations underlying epileptic seizures. In contradiction with this theory, several studies showed a lack of a functional synaptic inhibition between nRT neurons in the mouse. Here we provide the first compelling evidence of a functional intra-nRT GABAergic inhibition in the mouse, and we demonstrate that a feed-forward intra-nRT inhibition can be recruited by excitatory afferents. we expressed the ChR2-H134R construct in C57/BL6J mice specifically in glutamatergic thalamocortical relay (TC) neurons projecting to nRT. This allowed us to selectively stimulate TC axons with 473 nm light in horizontal thalamic slices. As expected, optical activation of TC axons led to an early excitation enhancing the action potential firing of nRT neurons due to TC-nRT glutamatergic projections. Notably, in a third of recorded nRT neurons, the early excitation was followed by an inhibition and a reduced firing in nRT neurons. The optically evoked inhibition in nRT neurons was blocked by GABAa receptor antagonists suggesting that this evoked inhibition resulted from GABAa- receptor-mediated feed-forward inhibition in TC-nRT-nRT pathway. Furthermore, GABAa receptor blockers, besides preventing the inhibition, reinforced the early excitation at TC-nRT synapses. Indeed, an early excitation that usually consisted of 1-5 action potentials switched, in presence of picrotoxin, to a robust burst firing underlined by NMDA receptors. This result demonstrates that the nRT-nRT inhibition shunts TC-nRT excitatory synaptic inputs. This study demonstrates for the first time the existence of a robust functional intra-nRT hyperpolarizing and shunting inhibition in thalamic slices from the mouse. Current studies ongoing in our laboratory aim at determining whether cortical inputs can also recruit intra-nRT inhibition and which specific concomitant or independent activity patterns in TC and cortico-thalamic pathways most efficiently recruit intra-nRT feed-forward GABAergic inhibition.

Disclosures: J. Paz: None. **J.R. Huguenard:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.07/E22

Topic: B.07. Synaptic Transmission

Support: Simons Foundation Grant 95395

NIMH NRSA F31MH084430

NIH Grant MH71739

Burnett Family Fund

Mosbacher Fund

Mathers Foundation

Title: Oxytocin activation of fast-spiking interneurons enhances hippocampal spike transmission

Authors: *S. F. OWEN[1], S. N. TUNCDEMIR[2], G. FISHELL[2], R. W. TSIEN[2];
[1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]NYU Neurosci. Inst., New York Univ., New York, NY

Abstract: Canonically, increasing inhibitory tone is expected to suppress neuronal responses to afferent stimuli. Here we show that the neuromodulator oxytocin selectively activates hippocampal fast-spiking interneurons. The resulting increase in inhibitory tone suppresses the spontaneous activity of CA1 pyramidal neurons, but surprisingly this is coupled with an enhancement of pyramidal cell responses to excitatory synaptic stimulation and a sharpening of evoked spike timing. The combination of a suppression of spontaneous activity paired with enhanced responses to evoked stimulation generates an improvement in network signal-to-noise, of possible importance to overall circuit performance. Activation of fast-spiking interneurons by the neuromodulator cholecystokinin or through the light-activated ion channel channelrhodopsin-2 yielded the same enhancement in evoked responses, confirming that this is a generally important network phenomenon. The selectivity of these interventions for fast-spiking interneurons sets them apart from other modulators, such as endocannabinoids, that target other interneuron classes in the hippocampus. The enhanced pyramidal cell spike throughput results from a suppression of disynaptic feed-forward inhibition that shifts the evoked excitatory-inhibitory balance. Paired whole cell recordings revealed that increasing fast-spiking interneuron activity induces a use-dependent depression at the fast-spiking cell to pyramidal cell synapse that

is necessary and sufficient to account for this reduction in feed-forward inhibition. On the other hand, excitatory transmission to pyramidal neurons and inhibitory neurons was directly assessed and appeared unaffected. These results delineate the action of oxytocin in the hippocampus, while also shedding light on a novel mechanism by which modulation of fast-spiking interneurons can modify hippocampal circuit activity.

Disclosures: S.F. Owen: None. **S.N. Tuncdemir:** None. **G. Fishell:** None. **R.W. Tsien:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.08/E23

Topic: B.07. Synaptic Transmission

Support: National Competence Center for Biomedical Imaging (NCCBI)

SystemsX.ch

Swiss National Science Foundation (SNSF)

European Research Council (ERC)

Title: *In vivo* measurement of synaptic transmission between identified neurons in layer 2/3 mouse barrel cortex

Authors: *A. PALA, C. C. H. PETERSEN;
Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: In order to obtain a causal and mechanistic understanding of how sensory information is processed in the neocortex, it is essential to measure the properties of synaptic transmission between neurons in living animals. Here, we use a combination of single-cell optogenetics and whole-cell electrophysiological recordings to control and record the in vivo activity of individual pre- and post-synaptic neurons. Two-photon targeted single-cell electroporation (Kitamura et al. 2008) is used to deliver DNA encoding channelrhodopsin-2 (ChR2) to a single excitatory neuron located in upper layer 2/3 of the mouse barrel cortex. After allowing sufficient time for ChR2 expression, the excitatory neuron can be optically driven to fire repetitive, reliable and time-locked single action potentials. Parvalbumin-expressing (PV) GABAergic neurons (identified genetically in PV-Cre/Lox-Stop-Lox-tdTomato transgenic mice) or somatostatin-expressing (SST) GABAergic neurons (identified genetically in SST-Cre/Lox-Stop-Lox-tdTomato transgenic mice) located in the vicinity of the ChR2-expressing neuron are then sequentially

recorded in the whole-cell configuration while the ChR2-expressing neuron is optically stimulated to fire action potentials. Using this method, preliminary analyses suggest that: i) the synaptic connectivity rate from excitatory neurons appears to be higher to PV neurons compared to SST neurons; ii) the unitary excitatory postsynaptic potential (uEPSP) amplitudes on average appear to be larger in PV neurons than in SST neurons; and iii) the uEPSP rise-time and half-width appear to be longer in SST neurons compared to PV neurons. We are now investigating short-term synaptic plasticity of these two synapses as well as the impact of spontaneous network activity on synaptic transmission.

Disclosures: A. Pala: None. **C.C.H. Petersen:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.09/E24

Topic: B.07. Synaptic Transmission

Support: ERC Starter Independent Grant, GA 260725 IRPHRCSTP

Title: Entorhinal-cortical HCN channel containing terminals originate from the forebrain

Authors: *M. M. SHAH, Z. HUANG;
Univ. Col. London, London, United Kingdom

Abstract: Hyperpolarization-activated Cation Non-selective (HCN) channels are highly expressed on hippocampal and cortical pyramidal cell dendrites. We have, though, recently demonstrated that HCN channels are also located on glutamatergic synaptic terminals that synapse onto medial entorhinal cortex (EC) layer III pyramids (1). Since afferents from cortical and non-cortical regions synapse onto EC layer III neurons, it is unknown which of these may contain presynaptic HCN channels. It is important to identify the neurons that contain these axonal channels as they inhibit glutamate release and are therefore important for regulating EC neuronal activity,
As a first step to address this question, we made entorhinal-cortical hippocampal slices from HCN1f/f,cre mice (in which HCN1 deletion is limited to the forebrain (2)) and their wild-type littermate controls. We recorded miniature excitatory synaptic currents (mEPSC) from EC layer III pyramidal neurons. We found that mEPSC frequency in HCN1f/f,cre mice was considerably enhanced compared to wildtype littermates. Further, consistent with our previous results using HCN1-/- mice in which HCN1 deletion was not restricted to the forebrain, bath application of the HCN channel inhibitor ZD7288 (15 μM) had little effect on HCN1f/f,cre mEPSC frequency.

Since HCN channels are expressed postsynaptically too and can alter EPSC shapes and summation, we performed FM1-43 imaging in slices using two-photon microscopy to directly measure synaptic release rates from individual terminals. Synaptic boutons in EC layer III were stably loaded with the fluorescent dye, FM1-43. Extracellular stimulation in the presence of the FM1-43 quenching, membrane impermeable dye, ADVASEP-7 was utilized to measure the rate of FM1-43 de-staining of terrminals (corresponding to exocytosis). We found that the rate and percentage of de-staining in HCN1f/f,cre slices was significantly greater than wildtypes. Additionally, whilst bath application of ZD7288 enhanced FM1-43 de-staining in wildtype slices, it had little effect in HCN1f/f,cre slices. These results suggest that the neurons containing pre-synaptic HCN channels are likely to be cortical in origin.
1) Huang, Z. et al. (2011) Nat Neurosci., 14, 478-486
2) Nolan, M.F., et al. (2004) Cell, 119, 719-32

Disclosures: M.M. Shah: None. **Z. Huang:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.10/E25

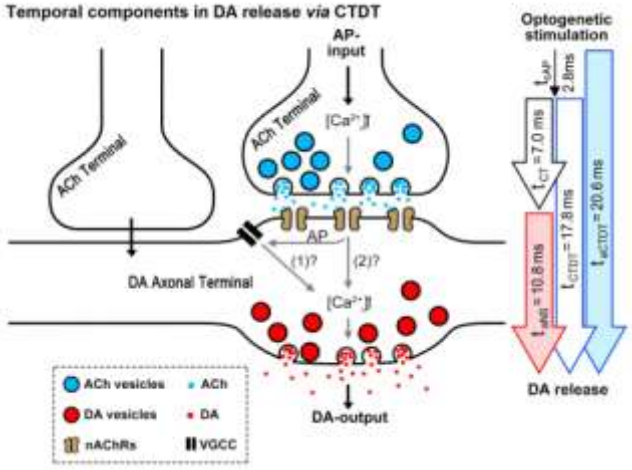
Topic: B.07. Synaptic Transmission

Title: Temporal components of a synaptic terminal-terminal transmission in dorsal striatum slices

Authors: *L. WANG, S. SHANG, L. ZHENG, S. TENG, F. ZHU, B. LIU, Q. WU, M. LI, W. LIU, H. XU, L. ZHOU, H. DOU, X. KANG, P. ZUO, C. WANG, S. WANG, Z. ZHOU;
Inst. of Mol. Medicine, Peking Univ., Beijing, China

Abstract: The fundamental striatal dopamine (DA) is released following stimulation by two pathways: the classic nigrostriatal pathway and the cholinergic "hijack" pathway through a cholinergic transmission-induced dopaminergic transmission (CTDT). The timing of synaptic transmission is critical in striatal circuits, because ms-latency change can reverse a synapse from LTP to LTD under DA-dependent manner. Here, we determined temporal components of CTDT in striatal slices. Following a light stimulation at room temperature, an optogenetic cholinergic interneuron fired an action potential (AP) with a delay of 2.8 ms. The subsequent CTDT mediated by nicotinic ACh receptors had a total latency of 17.8 ms, comprising 7.0ms for cholinergic transmission, and 10.8 ms for the downstream DA terminal release. The AP-induced replenishments of the “apparent vesicle pool” in the complex CTDT synapse contained 1 s

absolute refractory period. CTDT provides an example of unraveling the building blocks during a fundamental synaptic terminal-terminal transmission.



Disclosures: L. Wang: None. **S. Shang:** None. **L. Zheng:** None. **S. Teng:** None. **F. Zhu:** None. **B. Liu:** None. **Q. Wu:** None. **M. Li:** None. **W. Liu:** None. **H. Xu:** None. **L. Zhou:** None. **H. Dou:** None. **X. Kang:** None. **P. Zuo:** None. **C. Wang:** None. **S. Wang:** None. **Z. Zhou:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.11/E26

Topic: B.07. Synaptic Transmission

Title: NMDA receptor-dependent dendritic calcium spikes underlie hippocampal complex bursts *In vivo*

Authors: *C. GRIENBERGER, X. CHEN, A. KONNERTH;
Inst. for Neurosci., Munich, Germany

Abstract: Complex spike (CS) bursts are an essential feature of hippocampal CA1 pyramidal cell activity in vivo. They were reliably observed in rodents, rabbits and cats (e.g. Kandel and Spencer, 1961; Fujita, 1975; Ranck, 1973). Their relevance for place cell activity in exploring animals was recently directly established in vivo (Epszstein et al. 2011). Burst firing is considered to be critically involved in the induction of long-term potentiation (Sjöström and Nelson, 2002). Under in vitro conditions spontaneous burst firing in CA1 pyramidal cells is

much less pronounced than in vivo and consists mostly of 2-3 spikes. There is evidence that these short bursts are of intrinsic origin and involve sodium (Azouz et al., 1996) and/or calcium conductances (Metz et al., 2005). However, it remained unclear how the longer lasting CS bursts in vivo (4-8 spikes) are generated. Here, we combined in vivo two-photon microscopy and targeted whole-cell recordings to study CS bursts in mouse hippocampal CA1 pyramidal cells in vivo. In order to perform dendrite imaging, the hippocampus was exposed by removing a small portion of the covering cortical tissue. Spontaneous CS bursts were detected in all neurons tested (36/36). They consisted of high frequency spikes (groups of 4-8 spikes or more at 100-300 Hz) riding on a depolarizing wave (mean amplitude 32±7 mV). We found that the frequency of spontaneous CS bursts increased when increasing the membrane potential. CS bursts were completely abolished in neurons that were dialyzed with the NMDA receptor (NMDA-R) antagonist MK-801, revealing an essential role of NMDA-R activation for CS bursts in vivo. They were associated with large dendritic calcium signals in all basal dendrites. These calcium signals persisted when intracellularly blocking sodium channels with QX-314. They were associated with depolarizing waves (mean amplitude 30±7 mV) that were highly similar to those recorded during CS bursts under control conditions. Interestingly, the intracellular application of a broad calcium channel antagonist (D-890, Kovalchuk et al., 2000) blocked the depolarizing wave-associated multibranch dendritic calcium signals, but not local dendritic hotspot calcium signals corresponding to synaptic inputs sites. Instead, blocking NMDA-Rs abolished both multibranch as well as hot spot calcium signals. Thus, our results identify a novel type of NMDA receptor-dependent dendritic calcium spike in the basal dendrites of CA1 pyramidal cells, which is essential for CS burst generation in vivo.

Disclosures: C. Grienberger: None. **X. Chen:** None. **A. Konnerth:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.12/E27

Topic: B.07. Synaptic Transmission

Support: Deutsche Forschungs Gemeinschaft "Barrel Cortex Function" SNF-DFG FOR 1341

Deutsche Forschungs Gemeinschaft Exc 257 NeuroCure

European Research Council starting grant

Helmholtz Society

Title: *In vivo* monosynaptic excitatory transmission between layer 2 pyramidal neurons in mouse somatosensory cortex

Authors: *J.-S. JOUHANNEAU[1,2], A. L. DORRN[1,2], J. F. A. POULET[1,2];
[1]Dept. of Neurosci., Max-Delbrück Ctr. for Mol. Med. (MDC), Berlin-Buch, Germany;
[2]NeuroCure, Cluster of Excellence, Neurosci. Res. Ctr., Charité-Universitätsmedizin, Berlin, Germany

Abstract: Local, excitatory synaptic connections between glutamatergic pyramidal neurons underlie neocortical sensory processing and cognition, yet there is very limited data available on identified monosynaptic cortico-cortical connections in vivo. To characterise basic properties of excitatory monosynaptic connections in vivo, we used 2-photon microscopy to make visually targeted recordings from 2-4 neighbouring (<100um) layer 2 pyramidal neurons in somatosensory cortex of urethane anaesthetised P18-P21 wild type mice. Connections were tested with brief current injections to trigger single action potentials. Excitatory neurons were selected based on their current evoked firing pattern, the in vivo 2-photon fluorescent image and post-hoc biocytin stain. In vivo, cortical neurons show spontaneous subthreshold membrane potential changes, defining different cortical states. During hyperpolarized Downstates it was possible to record small amplitude unitary EPSPs triggered by single presynaptic action potentials. We find that excitatory synaptic connections between layer 2 pyramidal neurons in vivo are sparse, most are small amplitude and have kinetics that resemble excitatory connections found in previous brain slice studies. During depolarized Upstates, the connection amplitude is reduced. Therefore we show state dependent modulation of monosynaptic glutamatergic transmission in vivo.

Disclosures: J. Jouhanneau: None. **A.L. Dorrn:** None. **J.F.A. Poulet:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.13/E28

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS39395

Title: Inhibitory synaptic transmission between Purkinje neurons and neurons of the cerebellar nuclei in mice with the Angelman syndrome-linked mutation GABRB3 m-/p+

Authors: A. A. TURNOWCHYK[1], *I. M. RAMAN[2];
[1]Interdepartmental Neurosci. Program, Northwestern Univ., Evanston, IL; [2]Northwestern Univ., EVANSTON, IL

Abstract: The autism-linked gene, *GABRB3*, codes for the β3 subunit of the GABAA receptor and the maternal copy of the *Gabrb3* gene is lost in Angelman syndrome. The β3 subunit of the $GABA_AR$ has been shown to directly alter channel kinetics and influence the localization of $GABA_A$ receptors, both of which are predicted to change the speed and efficacy of fast inhibitory synaptic transmission. Inhibitory transmission plays a central role in the cerebellum, because Purkinje (Pkj) cells, the principal projection neurons of the cerebellar cortex, are GABAergic. In addition, the cerebellum is consistently disrupted in autism spectrum disorders, and *Gabrb3* m-/p+ mice show deficits in cerebellar behaviors. We therefore investigated the effects of the m-/p+ mutation on synaptic transmission from Pkj cells to their target neurons in the cerebellar nuclei (CbN) in cerebellar slices from P17-24 mice. We made whole cell patch clamp recordings from large projection CbN cells and evoked trains of IPSCs by stimulating Pkj axons at 100 Hz for 500 ms at 36°-37.5°C with synaptic excitation blocked by DNQX and CPP. In both littermate control (m+/p+) and m-/p+ neurons, IPSCs decayed with a fast time constant of ~1-2 ms and a variable slow component. We measured both the peak IPSC relative to the preceding baseline (the 'phasic' IPSC), as an indicator of presynaptic release, and the IPSC remaining just before each stimulus (the 'tonic' IPSC), as an indicator of postsynaptic kinetics. In m+/p+ cells, phasic IPSCs depressed only mildly, with the 50^{th} response at 78±18% of the 1^{st} (N=9). Likewise, in m-/p+ mice, the phasic IPSC stayed at 71±6% of the 1^{st} peak (N=13). In m+/p+ cells, the IPSCs did not decay fully between stimuli, so that the tonic current increased over the first 5 IPSCs, reaching 14 ± 3% of the 1^{st} IPSC peak and remaining at 10-13%. In contrast, in m/p+ cells, the tonic current increased only to 6±2% by the 5^{th} stimulus, and stayed at 5-7%. Thus, the β3 subunit regulates the kinetics of fast GABAergic IPSCs at this synapse. Next, we tested whether m-/p+ cells might have an enhanced ability to follow rapid trains of synchronous IPSCs, since the tonic IPSC is the primary factor suppressing CbN cell firing, and our recent work suggests that synchrony of Pkj cells can reduce tonic current and permit CbN cells to fire well-timed spikes between IPSCs. Unlike m+/p+ cells, which did not entrain perfectly to 100 Hz trains, m/p+ cells generated at least one spike after every synchronous IPSC, even at 100 Hz. These data support the hypothesis that faster IPSC kinetics as a consequence of reduced β3 expression result in a higher firing frequency in CbN cells during synchronous Pkj cell input.

Disclosures: A.A. Turnowchyk: None. **I.M. Raman:** None.

Poster

517. Synaptic Transmission: Synaptic Integration I

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.14/E29

Topic: B.07. Synaptic Transmission

Support: Dystonia Medical Research Foundation

Edward Mallinckrodt Jr. Foundation

National Science Foundation

Whitehall Foundation

Title: Abnormal cytoplasmic calcium dynamics in central neurons of a dystonia mouse model

Authors: *S. IWABUCHI, J.-Y. KOH, N. C. HARATA;
Dept. of Mol. Physiol. & Biophysics, Univ. of Iowa, Iowa City, IA

Abstract: Increased activities of cytoplasmic calcium and the excitatory neurotransmitter glutamate have been independently implicated in the pathophysiology of dystonia characterized by sustained involuntary muscle contractions. However, cellular-level evidence linking these two features is not available. Here we show that glutamate-dependent changes in neuronal calcium dynamics occur in a knock-in mouse model of DYT1 dystonia, the most common hereditary form of this disorder. Fluorescence-based analysis of the dynamics of cytoplasmic calcium concentration ($[Ca^{2+}]_c$) in cultured hippocampal neurons shows that electrical stimulation depolarizes the neurons and increases the dendritic $[Ca^{2+}]_c$, which then decays slowly to the pre-stimulus level. Whereas the peak amplitude of $[Ca^{2+}]_c$ was not affected, the decay period was prolonged in hippocampal neurons of heterozygous mice whose genotype reflects the human condition. We found that this effect was blocked by the antagonists of ionotropic glutamate receptors, and confirmed that glutamate receptors are present in these neurons. In contrast, no $[Ca^{2+}]_c$ abnormality was found in cultured striatal neurons of heterozygous mice that were composed of GABArgic medium spiny neurons and GABArgic interneurons. These results suggest that glutamate-dependent abnormality represents an important cellular phenotype of dystonia.

Disclosures: S. Iwabuchi: None. **J. Koh:** None. **N.C. Harata:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.15/E30

Topic: B.07. Synaptic Transmission

Support: Foundation Fighting Blindness

CIHR

Title: Electrical and chemical synapses drive fine-scale correlations in the retina

Authors: *A. J. MCLAUGHLIN[1], S. TRENHOLM[1], D. J. SCHWAB[2], G. B. AWATRAMANI[1];
[1]Univ. of Victoria, Victoria, BC, Canada; [2]Physics, Princeton Univ., Princeton, NJ

Abstract: Introduction
Throughout the CNS, neurons that are electrically coupled via gap junctions often exhibit fine-scale correlated activity (on a millisecond time scale). However, given the low conductance of gap junctions, it is unclear how they effectively drive correlated activity. Here we characterize the biophysical properties of electrical connections between superior coding directionally selective ganglion cells (DSGCs) in the retina and investigate how gap junctions cooperate with chemical synapses to promote synchronized activity.
Methods
Using 2-photon targeted whole-cell voltage-clamp techniques we recorded the physiological responses of DSGCs labeled in the Hb9 transgenic mouse retina.
Results
When DSGC subtypes coding all four cardinal directions were loaded individually with the gap junction permeable tracer Neurobiotin, only the Hb9::eGFP-labeled population exhibited homologous tracer coupling. Consistent with these anatomical findings, only superior coding DSGCs exhibited voltage-dependent feedback spikelets (when DSGCs were depolarized above 0 mV) that were sensitive to gap junction blockers. Recordings from pairs of neighboring Hb9+ DSGCs revealed that coupling was reciprocal, non- inactivating and relatively weak (<1 nS). Depolarization by direct current injections into individual DSGCs only led to subthreshold activity in neighboring cells, indicating that gap junction signals on their own cannot activate their neighbors. Nevertheless, when DSGCs were stimulated with stationary or moving spot stimuli (evoking mixed chemical and electrical synaptic activity), spike activity between neighboring cells exhibited robust correlations. Cross-correlograms computed from stimulus driven activity revealed sharp bimodal peaks at + 2ms with a trough centered around 0 ms. These were blocked by the gap junction antagonist 18-beta-glycyrrhetinic acid suggesting an obligatory role for reciprocal coupling. Fine-scale correlations were not present in pairs of other types of DSGCs with overlapping receptive fields but orthogonal directional preferences. Thus we conclude that both chemical and electrical synapses are required for generating correlated activity in the retina.
Conclusions
We identified a transgenic mouse line which labels a gap junction coupled population of superior

coding DS ganglion cells. This provides a novel tool for the study of electrical coupling between DSGCs. Our results indicate that electrical and chemical synapses cooperate to produce correlated activity between neighboring superior coding ON OFF DSGCs, which is expected to significantly impact information flow to higher centers.

Disclosures: A.J. McLaughlin: None. **D.J. Schwab:** None. **G.B. Awatramani:** None. **S. Trenholm:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.16/E31

Topic: B.07. Synaptic Transmission

Support: TEKES, The Human Spare Parts project 2011-2014

Title: Development of network connectivity in human pluripotent stem cell derived neural networks

Authors: *M. E.-L. MÄKINEN, L. YLÄ-OUTINEN, D. FAYUK, S. NARKILAHTI;
IBT, Univ. of Tampere / Biomeditech, Tampere, Finland

Abstract: Neural cells integrate into existing networks using similar mechanisms as newborn neural cells during fetal development (Jäderstad et al., 2010, Stephens et al 2011). During embryogenesis, the developing brain expresses a form of spontaneous synchronous network activity (Voigt et al., 2001). Similar activity develops spontaneously in the networks formed by human pluripotent stem cell (hPSC) derived neurons in vitro (Heikkilä et al., 2009). However, the cellular composition and the intercellular connections in spontaneously active neural networks remain unclear. Here, we studied the cellular components of network communication underlying the formation of early activity in hPSC derived neuronal networks.
hPSCs were differentiated to neurons as described earlier (Lappalainen et al., 2010). The analysis of functional cellular subpopulations and connections requires activity measurements. The activity of neural cell cultures is commonly measured with microelectrode arrays (MEAs) or calcium imaging. These two methods were combined with pharmacological studies to follow the spontaneous network activity and to dissect the mechanisms mediating activity during the maturation of the networks.
The early network activity was found to be mediated by gap junctions as well as by glutamatergic and GABAergic signalling. The amount of gap junction and GABA mediated signaling seemed to change during network maturation. In addition, the dependency of the

network activity from these mechanisms was different between differently derived networks. Furthermore, networks were found to contain subnetworks responding differently to pharmacological treatments.
The activity in hPSC derived neuronal networks is mediated by mechanisms similar to those occurring during the early brain development. The described pharmacological protocols seemed promising for studying differences in cellular composition and functional connections. Thus, human pluripotent stem cell derived neuronal cell cultures are a suitable in vitro platform for studying the integration of neurons into functional tissue.

Disclosures: M.E. Mäkinen: A. Employment/Salary (full or part-time):; University of Tampere / BioMediTech. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); The personel of IBT. **L. Ylä-Outinen:** None. **D. Fayuk:** None. **S. Narkilahti:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.17/E32

Topic: B.07. Synaptic Transmission

Support: NKTH grant (OMFB-01678/2009)

HAS "Lendulet" grant

Title: Powerful control of principal cell output by parvalbumin and cholecystokinin/CB1 cannabinoid receptor expressing interneurons in mouse basolateral amygdala

Authors: *J. VERES, G. A. NAGY, V. K. VERECZKI, N. HÁJOS;
Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary

Abstract: Synaptic inhibition powerfully controls the firing activity of principal cells (PCs) in the basolateral nucleus of the amygdala. However, it is currently unknown how the different inhibitory interneuron types contribute to the GABAA receptor-mediated synaptic transmission regulating the discharge of excitatory cells. In this work we tested the ability of interneurons expressing parvalbumin (PV-INs) or cholecystokinin and CB1 receptor (CCK/CB1-INs) to control the output properties of PCs in the basolateral amygdala.
We recorded from synaptically connected pairs of interneurons and PCs using in vitro whole-cell or perforated patch-clamp techniques. Amygdalar slices were prepared from PV-EGFP and CCK-DsRed transgenic mice. First, we recorded IPSCs and IPSPs in the postsynaptic PCs upon

evoking action potentials in the presynaptic interneurons and analyzed the basic kinetical properties of the synaptic transmission. Then we tested the ability of the interneurons to inhibit action potential generation in PCs evoked by somatically injected currents and to alter the timing of the output of their postsynaptic partner. We found that the majority of PV-INs and CCK/CB1-INs could veto PC firing and -depending on the timing of the inhibition- delayed the generation of the action potentials. When we discharged the PCs with electrically evoked EPSPs, we could observe a significant, but less robust inhibitory control of PC output by both cell types. During the recordings we labeled both the pre- and the postsynaptic cells for subsequent anatomical analysis and compared the physiological properties of the synapses with the corresponding anatomical features at the light and electron microscopic levels. We found that both types of interneurons contacted somatic and dendritic domains of PCs with similar distribution pattern, and that the location of the contacts correlated with various electrophysiological properties of the connections.
Although PV-INs and CCK/CB1-INs may play distinct roles in cortical network functions, these results suggest that under our circumstances both interneuron types can effectively control action potential generation in PCs. By regulating PC output these GABAergic cells are in the position to powerfully contribute to the formation and expression of fear memories in the basolateral amygdala.

Disclosures: J. Veres: None. **G.A. Nagy:** None. **N. Hájos:** None. **V.K. Vereczki:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.18/E33

Topic: B.07. Synaptic Transmission

Support: American Epilepsy Foundation

NIH grant 8P20GM103432-12

Title: Optogenetic investigation of axo-axonic inhibitory synapses within a cortical circuit

Authors: *X. WANG, Q.-Q. SUN;
Zoology and physiology 3166, Univ. of Wyoming, University Of Wyoming, WY

Abstract: GABAergic synapses provide an inhibitory tone to local circuits, thereby determining their final output. Among diverse GABAergic interneurons, axo-axonic synapses (AAS) provided by chandelier cells play a key role in the regulation of neural oscillation and

epileptogenesis. Chandelier cells provide a powerful control over the neural output of principal neurons by innervating the axon initial segments (AIS). However, little is known about their functional properties, due to technical challenges. Our recent work revealed that the piriform cortex (PC) inhibitory circuits are extremely unique. Virtually every pyramidal neuron in the PC area is innervated by robust AAS. This feature makes the PC the brain area with the highest density of AAS. In animal studies for temporal lobe epilepsy, the PC is the most prone to seizures and highly active chronic epileptic genesis foci within the limbic system. The unique anatomical and physiological features of the AAS make it an important component of the endogenous anti-epileptic circuit. Optogenetic tools, providing precise control of neural activities in a space and time specific fashion, have become the tools of choice for the study of neural circuits. This combined with in vitro slice electrophysiology, can shed light on the function of AAS innervations in the inhibitory network of the PC of mice. This lab has developed a convenient fiber optic approach to achieve subcellular laser stimulation at the AIS. After that, we used gramicidin-based perforated patch recordings to study the functional effects of AAS. Lastly, we induced "epileptic-form" activities and examined the effects of selective activation of AAS. We concluded that AAS have very transient veto power to neuronal excitability. Thus, techniques to selectively activate AAS may be used in treating neurological disorders such as temporal lobe epilepsy.

Disclosures: X. Wang: A. Employment/Salary (full or part-time):; University of Wyoming. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; American Epilepsy Foundation, NIH grant 8P20GM103432-12. **Q. Sun:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.19/E34

Topic: B.07. Synaptic Transmission

Support: KAKENHI (B) 24300091

Title: The hysteresis of evoked spike patterns in a cultured neuronal network depends on the strength of functional connections

Authors: *H. ITO, S. KUDOH;
Sch. of Sci. and Tech.,Kwansei Gakuin Univ., Sanda-City , Hyogo, Japan

Abstract: Cultured rat hippocampal neurons autonomously form a complex network on a multielectrodes array (MEA) dish, which is useful for analyzing network dynamics. Spontaneous activity starts to be observed from approximately 7 days in vitro (DIV). The structure of the network developed in order to form complex dynamics. We applied sequential electrical stimuli to the neuronal network. Shots of electrical stimuli were applied 4 times at a trial. Each of the first 2 stimuli, stim1 and stim2, were applied as a single stimulus with enough inter-stimulus interval. Last 2times, stim3 and stim4, were paired pulse stimulation with short ISI (stim4 with stim3 as pre-stimulation). The interval of stim3 and stim4 was set as 1 s, 2 s, 2.5 s, 5 s and 10 s. We found that electrical activity pattern by stim4 was affected by existence of stim3 in the case that interval between the stim3 and stim4 was within 2 s, in the case that the neuronal network was cultured for relatively long period (over 70 DIV). The hysteresis has not been observed in a network cultured for relatively short period (less 30 DIV). The origin of this difference is expected to be the differences in synaptic efficacy. To confirm the hypothesis, same experimental procedures were performed in the Mg2+-free Rec.Sol, which was expected to induce transient increase of synaptic efficacy by the activation of NMDARs and Ca2+channels. Under the raised synaptic efficacy, hysteresis was also observed in short period (aapproximately 30 DIV) cultures. Thus, synaptic efficacy controls the hysteresis of Network response. The hysteresis is generated depending on the internal state of the semi-artificial neuronal network on a MEA dish.

Disclosures: H. Ito: None. **S. Kudoh:** None.

Poster

517. **Synaptic Transmission: Synaptic Integration I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 517.20/E35

Topic: B.07. Synaptic Transmission

Support: NIH Grant DA026417

Title: Reuptake transporters limit dopamine but not noradrenaline pooling during autoreceptor feedback inhibition

Authors: N. A. COURTNEY, *C. FORD;
Physiol. and Biophysics, Case Western Reserve Univ., Cleveland, OH

Abstract: G-protein coupled receptors (GPCRs) are typically located extrasynaptically and activated by transmitter spillover. As such, slow synaptic potentials mediated by neurotransmitters acting via GPCRs classically signal through volume transmission. Whether

this holds true for all GPCRs remains unclear. Here, we compared metabotropic inhibitory post-synaptic currents (IPSCs) mediated by D2 dopaminergic receptors in the ventral tegmental area (VTA) with IPSCs mediated by alpha2 noradrenergic receptors in the locus coeruleus (LC). Both D2- and alpha2-receptors are GI coupled GPCRs that generate their IPSCs by causing the opening of G-protein coupled, inwardly rectifying potassium channels (GIRKs). Comparisons of D2- and alpha2-IPSCs evoked by a single extracellular stimulation revealed that alpha2-IPSCs were 250% slower to rise (10%-90% rise time), 400% longer in duration (half-width), and 625% more variable in decay time (standard deviation; alpha2-IPSC: n = 14, D2-IPSC: n = 17). These observations could not be explained by intrinsic receptor-GIRK coupling properties, differences in the timing of pre-synaptic exocytotic release, or dendritic filtering. The variability in the decay of alpha2-IPSCs significantly correlated to the amount of noradrenaline released as increasing the stimulation intensity driving release extended the duration of the alpha2-IPSC by 225% at maximum stimulations (n = 15 cells). This suggested pooling of noradrenaline in the extrasynaptic space prolonged alpha2-receptor transmission. D2-IPSCs, in contrast, had a consistent time course of decay regardless of the amount of transmitter released (n = 12 cells). Additional experiments determined that efficient reuptake through dopamine reuptake transporters (DATs) limited the pooling of dopamine to maintain the temporal fidelity of dopamine transmission. The function effects of the differences in clearance mechanisms across catecholamine synapses may determine the range and duration over which dopamine and noradrenaline can signal.

Disclosures: N.A. Courtney: None. **C. Ford:** None.**Poster**

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.01/E36

Topic: B.08. Synaptic Plasticity

Title: Augmented latent inhibition and lower seizure threshold in protein interacting with C kinase (PICK1) knock-out mice

Authors: *H. M. ARNOLD[1], P. YANG[1], S. GLASS[1], R. L. HUGANIR[2], K. RHODES[1], A. DUNNAH[1];
[1]Neurol. Res., Biogen Idec, CAMBRIDGE, MA; [2]Johns Hopkins Univ. Sch. of Med. and Howard Hughes Med. Inst., Baltimore, MD

Abstract: Protein interacting with C kinase (PICK1) plays a critical role in synaptic plasticity through the regulation of trafficking and posttranslational modification of interacting proteins. One such protein is metabotropic glutamate receptor 7 (mGluR7), which is widely expressed on

presynaptic terminals of neurons throughout the brain and is proposed to regulate neurotransmitter release. mGluR7-deficient mice displayed spatial working memory deficits and delayed extinction of conditioned fear, and also were hypersensitive to proconvulsive pentylenetetrazole (PTZ) (Holscher et al., 2004, Callaerts-Vegh et al.k, 2006, Sansig et al., 2001). Both learning and memory deficits and convulsant effects could be replicated by uncoupling PICK1 from mGluR7a through mutation of PDZ-ligand motif of mGluR7a, which suggested that the mGluR7a-PICK1 interaction is essential for mGluR7a-associated receptor signaling and targeting (Bertaso F, et al., 2008; Zhang C, et al., 2008). In the present study we further examined the role of PICK1 in synaptic plasticity by examining the behavior of PICK1 knockout (KO) mice in two paradigms; latent inhibition of conditioned fear and the PTZ-induced seizure threshold test. Homologous and heterologous PICK1 KO mice and their wild-type littermates were tested at six months of age. Latent inhibition (LI) is believed to share a common molecular requirement as fear extinction and was tested in this study by fear pairing of white-noise and foot shock. LI refers to the observation that animals presented with non-reinforced pre-exposure to a stimulus (white noise) will acquire a lower level of conditioned fear relative to animals without the stimulus pre-exposure. LI was observed in the wild-type animals as expected in this experiment. PICK1 KO mice showed an even greater effect of the pre-exposure or enhanced latent inhibition relative to the wild-type mice. Seizure threshold was assessed in the PTZ-induced seizure model, and PICK1 KO mice exhibited a lower seizure threshold compared to wild-type animals. Taken together these results are consistent with the literature that indicates that PICK1 is crucial for mGluR7-associated synaptic plasticity.

Disclosures: H.M. Arnold: A. Employment/Salary (full or part-time):; Neurology Research, Biogen Idec Inc, Weston, Massachusetts, USA. **P. Yang:** A. Employment/Salary (full or part-time):; Biogen Idec. **S. Glass:** A. Employment/Salary (full or part-time):; Biogen Idec. **K. Rhodes:** A. Employment/Salary (full or part-time):; Biogen Idec. **A. Dunnah:** A. Employment/Salary (full or part-time):; Biogen Idec. **R.L. Huganir:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.02/E37

Topic: B.08. Synaptic Plasticity

Title: Pharmacological disruption of PICK1-GluR2 interaction constitutes a novel target for neurological diseases associated with synaptic dysfunction

Authors: *C. C. BANOS[1], T. R. CHAN[1], F. JOW[1], E. Y. S. LIN[1], M. S. BRENNAN[1], R. L. HUGANIR[2], K. J. RHODES[1], R. H. SCANNEVIN[1], M. WITTMAN[1], K. M. GUCKIAN[1], A. W. DUNAH[1];
[1]Neurol. Res., Biogen Idec, Cambridge, MA; [2]Johns Hopkins Univ. Sch. of Med. and Howard Hughes Med. Inst., Baltimore, MD

Abstract: Altered neuronal synaptic plasticity, which is regulated by glutamate receptor trafficking, including calcium homeostasis are emerging pathophysiological features of several neurodegenerative and neuropsychiatric diseases. Changes in the trafficking of AMPA receptors can alter intracellular calcium levels and signaling at synapses. Here, we report that PICK1 plays an essential role in regulating the trafficking of GluR2 AMPA receptors in order to maintain synaptic plasticity and calcium homeostasis. We found that PICK1 inhibitors stabilize synaptic GluR2 receptors resulting in decreased AMPA-mediated calcium influx in cultured primary hippocampal neurons. Additionally, neurons lacking the PICK1 protein also show significant stabilization of GluR2 at synapses as well as a reduction in AMPA-mediated calcium influx. Moreover, PICK1 inhibitors inhibited long term depression in hippocampal slices. These findings suggest that pharmacological disruption of PICK1-GluR2 interaction can stabilize AMPA receptors at synaptic sites resulting in efficient preservation of synaptic plasticity and structural integrity. Therefore, PICK1-GluR2 pathway constitutes a potential therapeutic target for the development of novel treatments for neurological diseases.

Disclosures: C.C. Banos: A. Employment/Salary (full or part-time):; Biogen Idec. **T.R. Chan:** A. Employment/Salary (full or part-time):; Biogen Idec. **F. Jow:** A. Employment/Salary (full or part-time):; Biogen Idec. **E.Y.S. Lin:** A. Employment/Salary (full or part-time):; Biogen Idec. **M.S. Brennan:** A. Employment/Salary (full or part-time):; Biogen Idec. **R.L. Huganir:** None. **K.J. Rhodes:** A. Employment/Salary (full or part-time):; Biogen Idec. **R.H. Scannevin:** A. Employment/Salary (full or part-time):; Biogen Idec. **M. Wittman:** A. Employment/Salary (full or part-time):; Biogen Idec. **K.M. Guckian:** A. Employment/Salary (full or part-time):; Biogen Idec. **A.W. Dunah:** A. Employment/Salary (full or part-time):; Biogen Idec.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.03/E38

Topic: B.08. Synaptic Plasticity

Support: NSFC81100822

NFSC81271209

NFSC81070873

Ningbo Key Science and Technology Project(2011C51006),

Title: Soluble amyloid β-enhanced LTD in rat dentate gyrus of hippocampus is mGluR1/5-dependent and involves activation of p38MAPK, STEP and caspase-3

Authors: *L. CHANG, X. CHEN, R. LIN, J. HU, Q. WANG;
Physiol., Ningbo University, Med. Sch., Zhejiang, China

Abstract: Accumulation of amyloid beta (Aβ) protein is a key pathogenic element of Alzheimer's disease. It is reported that the Aβ-induced impairments in synaptic plasticity (LTP and LTD) coincides with memory decline and dementia. Although Aβ-induced inhibition of hippocampal long-term potentiation (LTP) has been intensively investigated, the underlying mechanism of Aβ-enhanced long-term depression (LTD) is not clear. Here, we report that acute exposure of rat hippocampal slices to soluble Aβ enhances LTD induced by sub-threshold low frequency stimulation (1Hz for 3 min, 180 pulses) in granule cells of dentate gyrus. Application of LY341495 (a non-selective Group I/II mGluR antagonist) completely block Aβ-enhanced LTD, whereas D-AP5 (a not selective NMDAR antagonist) has no effect on Aβ-enhanced LTD compared with untreated controls. In addition, Aβ-enhanced LTD is occluded by pre-application of 5-dihydroxyphenylglycine (DHPG), a Group1 mGluR (mGluR1/5) agonist, suggesting Aβ-enhanced LTD depends on mGluR1/5 but not NMDAR. We also report here that p38 mitogen-activated protein kinase (p38MAPK) inhibitor SB203580 and postsynaptic protein tyrosine phosphatase (PTP) inhibitors phenylarsine oxide (PAO) and sodium orthovanadate prevent the inhibitory effect of Aβ on LTD. The possible PTPs involved in Aβ-enhanced LTD could be striatal-enriched protein tyrosine phosphatase (STEP) because Aβ-enhanced LTD occludes a small LTD induced by STEP activator MG132 alone. Application of either non-selective caspase inhibitor Z-VAD-FMK or caspase-3 selective inhibitor Z-DEVD-FMK prevents Aβ-enhanced LTD, suggesting capase-3 is also necessary for the facilitatory effect of Aβ on LTD. However, neither tumor necrosis factor-α converting enzyme (TACE) inhibitor TAPI-2 nor mammalian target of Rapamycin (mTOR) inhibitor Rapamycin prevents the enhancement of Aβ on LTD. Therefore, we conclude that soluble Aβ enhances LTD in hippocampal dentate gyrus region, and the facilitatory effect of Aβ on LTD depends on mGluR1/5, p38MAPK, STEP and caspase-3 activation.

Disclosures: L. Chang: None. **X. Chen:** None. **J. Hu:** None. **R. Lin:** None. **Q. Wang:** None.

Poster

518. Long-Term Depression (LTD)

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.04/E39

Topic: B.08. Synaptic Plasticity

Support: the Japan Society for the Promotion of Science to A. O. (24650207)

Title: Asymmetric dendritic spine dynamics in the apparently symmetric long-lasting synaptic plasticity phenomena after repeated LTP/LTD inductions

Authors: S. HASEGAWA, Y. OE, K. TOMINAGA-YOSHINO, *A. OGURA;
Osaka Univ., Suita/Osaka, Japan

Abstract: Memory is one of the most important functions of the brain. Instantaneously acquired memory becomes long-lasting through the process called consolidation. But the cellular mechanisms of consolidation remain unclear, since the cellular correlate of consolidation is elusive. A currently prevailing view assumes that late-phase LTP accompanying morphological changes in dendritic spines is the in vitro reproduction of consolidation, but longevity of those changes is uncertain. We previously showed in the organotypic slice cultures of the rodent hippocampus that three repeated inductions of LTP and LTD by chemical means led to slowly developing long-lasting synaptic enhancement and suppression coupled with synapse formation and elimination, respectively. Naming these phenomena RISE (Repetitive LTP-Induced Synaptic Enhancement) and LOSS (LTD-repetition-Operated Synaptic Suppression), we propose these as the in vitro reproduction of memory consolidation suitable for the analysis of the cellular mechanisms of consolidation.
Our previous studies showed that the increase in net spine number in RISE is resulted from 2-step processes; 1) increased spine dynamics after the third LTP (increase in rates of both spine generation and retraction) followed by 2) earlier returning of the retraction rate to its basal level. In the present study we examined whether or not LOSS would follow symmetric 2-step processes. Using sequential confocal imaging (1-day or 5-day intervals) on the slice cultures from Thy1-YFP H-line transgenic mice that express YFP in the CA1 pyramidal neurons, we found that three inductions of chemical LTD by DHPG led to an increased rate of spine retraction leaving the rate of generation uninfluenced, resulting a decrease in net spine number. Single induction of LTD caused no apparent changes in both rates as judged from 1-day interval imaging. We showed previously that the spine number increase in RISE occurred preferentially in the dendritic segments having low pre-existing spine density. In LOSS, however, the spine number decrease relatively homogeneously irrespective to pre-existing spine density. These results reveal that RISE and LOSS proceed non-symmetrically, although the effects are apparently symmetric.

Disclosures: S. Hasegawa: None. **Y. Oe:** None. **K. Tominaga-Yoshino:** None. **A. Ogura:** None.

Poster

518. Long-Term Depression (LTD)

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.05/E40

Topic: B.08. Synaptic Plasticity

Support: Division of Intramural Clinical and Basic Research of the National Institute on Alcohol Abuse and Alcoholism, US National Institutes of Health

Title: Effects of a clinically-used opioid analgesic on dissociable forms of long-term depression in the dorsal striatum

Authors: *B. K. ATWOOD[1], D. KUPFERSCHMIDT[2], W. XIONG[2], D. LOVINGER[2];
[1]LIN, [2]NIAAA, Rockville, MD

Abstract: The dorsal striatum is a brain region that is critically involved in action selection and habit formation and as such plays an important role in addiction to drugs of abuse. Opioid peptides, their target receptors and the peptidase enzymes that are responsible for terminating their actions are abundantly expressed in this brain region. Many components of this endogenous opioid system are modulated by opioid analgesics as well as other drugs of abuse. We have previously demonstrated that a brief exposure to mu opioid receptor agonists produces long-term depression (OP-LTD) of excitatory inputs on to medium spiny neurons in the dorsal striatum. In addition we have shown that endogenously released opioid peptides are also able to induce OP-LTD. Interestingly these endogenously released opioids also exert their effects through mu and other opioid receptors. We thus explored whether agonists for other opioid receptors could also promote OP-LTD of glutamatergic transmission in the dorsal striatum. We also sought to determine what effect a commonly abused prescription painkiller, oxycodone, would have on plasticity mediated by these other opioid receptors as well as cannabinoid receptors. We found that agonists of mu and delta opioid receptors could each induce OP-LTD through their respective target receptors. Despite each receptor being able to induce OP-LTD upon activation, the two receptors were not able to occlude one another's effects. Furthermore, mu OP-LTD was mutually occlusive with endocannabinoid-dependent LTD, whereas delta OP-LTD was not. These data suggest that the two receptors are acting at different synapses or via distinct mechanisms. A single in vivo exposure to the opiate analgesic, oxycodone, disrupts the expression of mu OP-LTD and endocannabinoid LTD, but not delta OP-LTD. This disruption lasted for 2 days and was paralleled by a performance deficit in a rotarod training paradigm. These data demonstrate a novel form of long-lasting synaptic plasticity in the dorsal striatum that

is induced by a brief exposure to opioid peptides that has relevance for the consequences of opiate analgesic use.

Disclosures: B.K. Atwood: None. **D. Kupferschmidt:** None. **W. Xiong:** None. **D. Lovinger:** None.

Poster

518. Long-Term Depression (LTD)

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.06/E41

Topic: B.08. Synaptic Plasticity

Support: WCI program, NRF of Korea

Global Ph.D. Fellowship, NRF of Korea

Title: Role of DGKζ in the regulation of PKCα activity and cerebellar Purkinje cell synapses

Authors: *D. LEE[1,2], K. KIM[2], Y. KIM[1], Y. YAMAMOTO[1], E. KIM[2], K. TANAKA-YAMAMOTO[1];
[1]Ctr. for Functional Connectomics, KIST, Seoul, Korea, Republic of; [2]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: Long-term synaptic plasticity is triggered by synaptic stimulation, and activation of many signals is implicated in such synaptic plasticity, so that molecules working negatively on these signals must control signaling activities and synaptic plasticity. In cerebellar Purkinje cells, activation of protein kinas C (PKC), specifically PKCα, is well known to be one of key factors for triggering long-term synaptic depression (LTD). However, the negative regulators of PKC are not yet investigated. Diacylglycerol kinase ζ (DGKζ), which is strongly expressed in Purkinje cells, has been reported in a study using HEK293 cells to negatively regulate PKCα via binding with PKCα and metabolizing diacylglycerol, a PKCα activator. Further, our previous study demonstrated that DGKζ can be targeted to the excitatory synapses by interacting with PSD-95 family proteins (EMBO J, 2009, 28:1170). These studies raise a possibility that DGKζ regulates postsynaptic PKCα activation and consequently cerebellar LTD. In this study, we tested the role of DGKζ in the cerebellar LTD by using DGKζ knockout mice. First of all, we looked at the morphology of Purkinje cells. Although the Sholl analysis showed that distal dendritic trees in DGKζ knockout mice are more complex than those in their wild-type littermates, densities of dendritic spines were unaltered, so that deficiency of DGKζ seems to have less impact on synaptic morphology. We then recorded synaptic transmission at synapses between parallel

fibers and Purkinje cells. Surprisingly, LTD was blocked in the DGKζ knockout mice. It is unlikely that increase in basal PKCα activation caused by deficiency of DGKζ already induced LTD and that further induction of LTD was occluded, because basal synaptic transmission was unaltered in the DGKζ knockout mice. To investigate the PKCα activation, we used chemical LTD stimulation and observed translocation of endogenous PKCα from cytosol to the plasma membrane in Purkinje cell soma. This analysis revealed that PKCα activation is indeed enhanced in DGKζ knockout mice. One possible interpretation of these results is that hyperactivation of PKCα caused by deficiency of DGKζ leads to the impairment of LTD induction. In this case, this study brings up an idea that signals need to be precisely regulated via balancing between positive and negative regulators for triggering LTD. This idea is in part consistent with the leaky integrator property of LTD induction described in our previous study (Neuron, 2007, 54:787).

Disclosures: D. Lee: None. **K. Kim:** None. **Y. Kim:** None. **Y. Yamamoto:** None. **E. Kim:** None. **K. Tanaka-Yamamoto:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.07/E42

Topic: B.08. Synaptic Plasticity

Support: NIDA grant K01 DA21699

NIDA grant R01 DA32701

Title: Neurotensin induces long-term depression of dopamine D2 receptor-mediated inhibitory postsynaptic currents in substantia nigra dopamine neurons

Authors: *E. PICCART[1], M. J. BECKSTEAD[2];
[2]Physiol., [1]UTHSCSA, San Antonio, TX

Abstract: Midbrain dopamine (DA) neurons express high levels of D2-type autoreceptors ($D2R_{auto}$) on their cell bodies and dendrites. Iontophoretic application of DA or electrically evoked release of DA activates these autoreceptors and produces an outward current or an inhibitory postsynaptic current (IPSC), respectively. Prolonged activation of $D2R_{auto}$ induces long-term depression (LTD) of DA-mediated currents, an effect that is blocked by chelating calcium postsynaptically. Midbrain DA neurons receive dense innervation from neurotensin (NT) fibers. Activation of the predominant NT receptor 1 (NTR1) antagonizes D2 DA receptors via allosteric receptor/receptor interactions as well as second messenger signaling cascades. For

instance, NTR1 activation is shown to cause $D2R_{auto}$ internalization through PKC activation. Here, we addressed the effect of NT on $D2R_{auto}$-mediated currents using patch clamp electrophysiology in substantia nigra pars compacta DA neurons. Bath application of the active fragment NT_{8-13} (30, 100, 300 nM) depressed outward currents evoked by iontophoretic application of DA. This depression only persisted over time (30 min) when intracellular calcium was chelated using a high concentration of BAPTA (10 mM) in the pipette. Bath perfusion of the PKC-inhibitor staurosporine (1 M) did not affect NT-induced depression of outward currents. Bath application of NT_{8-13} also induced LTD of $D2R_{auto}$-mediated IPSCs, and this occurred independent of postsynaptic calcium chelation. We thus show a novel form of NT-induced synaptic plasticity in midbrain DA cells that appears to be independent of available intracellular calcium. The net result of this NT-induced depression of an inhibitory signal would be an increase in the excitability of DA neurons which could subsequently affect DA-mediated behaviors and disorders.
This work is supported by NIDA grant K01 DA21699 (M.J.B) and R01 DA32701 (M.J.B).

Disclosures: E. Piccart: None. **M.J. Beckstead:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.08/E43

Topic: B.08. Synaptic Plasticity

Support: NIAG032320

NIGMS, GM080202

Title: The role of X-linked mental retardation protein, BRAG1, in synaptic function

Authors: *J. C. BROWN[1], L. ZHONG[1], A. PETERSEN[1], R. WALIKONIS[2], N. Z. GERGES[1]; [1]Cell Biol, Neurobio. & Anat., Med. Col, Wisconsin, Milwaukee, WI; [2]Physiol. and Neurobio., Univ. of Connecticutt, Storrs, CT

Abstract: Learning disorders often result from disrupted AMPA receptor trafficking. X-linked mental retardation (XLMR) is a developmental disorder characterized by significant limitations in cognitive function, and the ability to perform basic daily tasks. Recently, mutations of a post-synaptic density (PSD) protein, BRAG1, were identified as a cause of XLMR (Shoubridge et al., 2010).
Here we report that BRAG1 is sufficient to increase the synaptic expression of recycling GluA2-

containing AMPA receptors in hippocampal slice cultures. This enhancement was blocked with an interfering peptide designed to prevent interactions with the PDZ-binding domain. Moreover, a c-terminal truncation lacking the PDZ-binding domain failed to produce an increase, indicating that BRAG1 PDZ interactions facilitate synaptic GluR2 expression.
BRAG1 guanine nucleotide exchange factor (GEF) activity was shown by Shoubridge et al. to be impaired in XLMR patients. We report that BRAG1 GEF activity is required for long-term depression. In fact, blocking BRAG1-GEF activity drastically increases AMPAR-mediated synaptic transmission after prolonged expression, suggesting AMPAR accumulation over time in the absence of GEF-mediated internalization. Together, these results indicate a critical role in synaptic function for BRAG1 involving both insertion and removal of AMPARs, providing new insight into mechanisms underlying learning disabilities.

Disclosures: J.C. Brown: None. **L. Zhong:** None. **A. Petersen:** None. **R. Walikonis:** None. **N.Z. Gerges:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.09/E44

Topic: B.08. Synaptic Plasticity

Support: ES100221

Title: Regional variations in phosphodiesterase and adenylyl cyclase activity contribute to the differential expression of adenosine A1 receptor-mediated synaptic plasticity in hippocampal areas CA1 and CA2

Authors: *D. A. CARUANA, S. M. DUDEK;
NIEHS/NIH, Research Triangle Park, NC

Abstract: Adenosinergic signaling in the hippocampus regulates both synaptic function and behavior. We have shown previously that adenosine A1 receptors (A1Rs) mediate bidirectional changes in synaptic efficacy at Schaffer collateral (SC) inputs to CA2 that are not seen at CA1 synapses. Such differential sensitivity to A1R agonists and antagonists across hippocampal subfields likely reflects differences in both the regional and developmental expression of A1Rs and associated downstream effectors. Indeed, although staining for A1R protein is uniform across the rodent hippocampus early in development, it changes dramatically after postnatal day 28, with staining increasing in CA2 and decreasing in CA1 as animals mature. Our data using stimulation of A1Rs support this developmental pattern of staining: we have shown that low

concentration of A1R agonists induce long-term depression (LTD) in SC inputs to both CA1 and CA2 in slices obtained from juvenile rats, but only in CA2 in adult slices. Surprisingly, effects of A1R antagonists on synaptic responses do not follow a similar developmental pattern: inhibiting A1Rs potentiates EPSCs in CA2, but not in CA1, regardless of age. To determine the key intracellular signals underlying these regional differences in A1R-mediated synaptic plasticity, we recorded whole-cell EPSCs from pyramidal neurons in juvenile rat CA2 and CA1 evoked by stimulating the SCs. Bath-application of the selective A1R agonist CCPA (100nM) for 5 min induced LTD of EPSCs in both CA2 and CA1 that persisted for 60 min. The CCPA-mediated depression relied on neither NMDA receptors nor p38 MAP kinase activation, and did not require synaptic stimulation for its induction. We next tested whether inhibiting cAMP degradation would prevent LTD induced by CCPA. Surprisingly, the phosphodiesterase (PDE) inhibitor rolipram (10μM) blocked A1R-mediated LTD in CA1, but not in CA2, suggesting that the PDE subtypes or basal PDE activity levels in CA1 differ considerably from those in CA2. We have noted that several PDE isoforms are differentially expressed in CA1 or CA2 (e.g. Pde8b in CA1). Finally, we examined whether the selective potentiation in the juvenile CA2 is due to downstream targets of A1Rs, including some adenylyl cyclases that are highly expressed in CA2 (e.g., adcy1, 5 or 6). We found that forskolin (10μM), which stimulates adenylyl cyclases directly, potentiated EPSCs in CA2, but not in CA1. Together, these data indicate that the heightened sensitivity of the juvenile CA2 in response to A1R antagonists is a reflection of the differential expression of both adenylyl cyclases and phosphodiesterases rather than enrichment of the A1 receptors.

Disclosures: D.A. Caruana: None. **S.M. Dudek:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.10/E45

Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH078009

Title: Chronic stress impairs α_1-adrenergic receptor-induced endocannabinoid ltd in the dorsal raphe nucleus

Authors: *S. HAJ-DAHMANE, J. WANG, R.-Y. SHEN;
SUNY @ Buffalo, BUFFALO, NY

Abstract: In the dorsal raphe nucleus (DRn), activation of alpha 1-adrenergic receptors (α_1-ARs) exerts a tonic activation of DRn serotonin (5-HT) neurons and plays a crucial role in regulating arousal and the behavioral responses to stress. However, the precise effects of these receptors on glutamatergic synaptic transmission to DRn 5-HT neurons and how chronic exposure to stress alters α_1-ARs-induced modulation of the synaptic function in the DRn remain unknown. In the present study, we examined the impact of the activation of α_1-ARs on the strength and plasticity of glutamate synapses of putative DRn 5-HT neuron in control rats and after chronic exposure to restraint stress. We found that in control animals, activation of α_1-ARs elicited a postsynaptic membrane depolarization/inward current and long-term depression (LTD) of excitatory synaptic transmission onto putative DRn 5-HT neurons. The α_1-AR-LTD revealed that it was mediated by a decrease in glutamate release and signaled by retrograde endocannabinoid (eCB) messengers. In addition , we found that exposure to chronic (7 days) restraint stress profoundly reduced the magnitude of α_1-AR-LTD, without altering the amplitude of α_1-receptor-induced inward current. The stress-induced impairment of α_1-AR-LTD was not mediated by an alteration α_1-AR function but rather by a down regulation of presynaptic CB1 receptors. Together, our results demonstrate that noradrenergic tone in DRn control synaptic plasticity by recruiting eCB signaling. They also provide the first evidence that chronic stress alters eCB-mediated synaptic plasticity in the DRN which could represent a potential mechanism underlying stress homeostasis.

Disclosures: S. Haj-Dahmane: None. **J. Wang:** None. **R. Shen:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.11/E46

Topic: B.08. Synaptic Plasticity

Support: NSF Grant EF-1137897

NIH Grant HD-G12MD007591

NSF Grant HDR-09323339

Title: Effects of anomalous diffusion on synaptic plasticity

Authors: *T. MARINOV[1], F. SANTAMARIA[2];
[1]Univ. of Texas at San Antonio, San Antonio, TX; [2]UTSA Neurosciences Inst., Univ. of Texas, San Antonio, TX

Abstract: We have experimentally and computationally demonstrated that intracellular dendritic diffusion of soluble signals can be anomalous in Purkinje and pyramidal cells. Dendritic spines cause anomalous diffusion by acting as traps that transiently capture diffusing molecules. Anomalous diffusion is characterized by a mean square displacement (msd) of a diffusing particle that follows a power law (msd ~ t^a). Here we study the role of anomalous diffusion in the spatial and temporal expression of long term depression (LTD) of the parallel fiber to Purkinje cell synapse. LTD is mediated by a positive feedback loop involving [Ca2+], PKC and MAPK. While [Ca2+] does not undergo anomalous diffusion, PKC and MAPK are large proteins susceptible to molecular trapping by spines. Using our recently developed fractional integration toolbox we solve the anomalous diffusion equation based on fractional calculus. We have implemented a simplified LTD model of coupled fractional diffusion-reaction equations for [Ca2+], PKC and MAPK with non-constant diffusion coefficients and reaction rates for various fractional orders and spatial and temporal distributions. Fractional diffusion results in longer activation times for the PKC-MAPK positive feedback loop. Once activated, PKC and MAPK stay activated longer, implying a lower [Ca2+] activation threshold. Thus, anomalous diffusion of signaling molecules along dendrites could affect the sensitivity of expression of synaptic plasticity.

Disclosures: T. Marinov: None. **F. Santamaria:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.12/E47

Topic: B.08. Synaptic Plasticity

Support: NIH Grant DC008984

Title: Activation of synaptic group II mGluRs induces long-term depression at GABAergic synapses in CNS neurons

Authors: *Y.-W. LIU, Z.-Q. TANG, W. SHI, E. DINH, W. HAMLET, R. CURRY, Y. LU; Northeast Ohio Med. Univ., Rootstown, OH

Abstract: Metabotropic glutamate receptor (mGluR)-dependent homosynaptic long-term depression (LTD) has been studied extensively at glutamatergic synapses in the CNS. However, much less is known about heterosynaptic long-term plasticity induced by mGluRs at inhibitory synapses. Here we report that pharmacological or synaptic activation of group II mGluRs (mGluR II) induces LTD at GABAergic synapses in neurons of the chicken cochlear nucleus.

Coefficient of variation and failure rate analysis revealed that the LTD was expressed presynaptically. The LTD requires presynaptic spike activity, but does not require the activation of NMDA receptors. The classic cAMP-dependent protein kinase A signaling is involved in the transduction pathway. Interestingly, mGluR II did not modulate the excitatory glutamatergic transmission of NM neurons. Blocking mGluR II increased spontaneous GABA release, indicating the presence of tonic activation of mGluR II by ambient glutamate. Synaptically released glutamate induced by electrical stimulations that concurrently activated both the glutamatergic and GABAergic pathways resulted in significant and constant suppression of GABA release at various stimulus frequencies (3.3, 100, and 300 Hz). Strikingly, low frequency stimulation (1 Hz, 15 min) of the glutamatergic synapses induced heterosynaptic LTD of GABAergic transmission without altering glutamatergic transmission, and the LTD was blocked by mGluR II antagonist, indicating that activation of synaptic mGluR II induced the LTD. This novel form of long-term plasticity in the avian auditory brainstem may play a role in the development as well as in temporal processing in the sound localization circuit.

Disclosures: Y. Liu: None. **Z. Tang:** None. **W. Shi:** None. **E. Dinh:** None. **W. Hamlet:** None. **R. Curry:** None. **Y. Lu:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.13/E48

Topic: B.08. Synaptic Plasticity

Support: FRAXA Research Foundation grant 2013

Title: Intracellular mechanisms responsible for serotonin-mediated reversal of metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD) in wild-type and Fmr1 KO mouse hippocampus

Authors: L. T. COSTA[1], C. M. BONACCORSO[2], S. A. MUSUMECI[2], M. V. CATANIA[3,2], *L. CIRANNA[4];
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Abstract: We have shown that activation of serotonin type 7 (5-HT7) receptors prevents metabotropic glutamate receptor-mediated endocytosis of AMPA receptors and subsequent long-term depression (mGluR-LTD) in the hippocampus of wild-type (WT) and Fmr1 KO mice, a

mouse model of Fragile X Syndrome (FXS) in which mGluR-LTD is abnormally enhanced (Costa et al., Biol. Psych. 2012, 72:924-933). 5-HT7 receptors are classically coupled to stimulation of adenylate cyclase and protein kinase A (PKA) but other signalling pathways have been suggested, including the kinases Akt and glycogen synthase kinase 3 (GSK3).
To identify the intracellular mechanisms involved in 5-HT7-mediated reversal of mGluR-LTD, we recorded AMPA receptor-mediated excitatory post-synaptic currents ($EPSC_{AMPA}$) from CA1 pyramidal neurons under patch clamp on hippocampal slices from WT mice. Application of the group-I mGluR agonist DHPG induced a long-term depression (mGluR-LTD) of $EPSC_{AMPA}$. When DHPG application was followed by application of either 5-HT or 8-OH DPAT (a mixed 5-HT1A/5-HT7 agonist), DHPG-induced mGluR-LTD was completely reversed. 5-HT-mediated reversal of mGluR-LTD persisted in the presence of intracellular cAMPS-Rp (a cAMP analogue which binds PKA without activating it) and was not mimicked by 8-Br-cyclic AMP.
When lithium was included in the intracellular solution at a concentration (20 mM) that inhibits GSK3, the amount of DHPG-induced mGluR-LTD was not significantly different from control conditions, but the reversal effect of 8-OH-DPAT application on mGluR-LTD was blocked. These results indicate that reversal of mGluR-LTD by 5-HT7 receptor activation is not mediated by the cAMP/PKA pathway and likely involves inhibition of GSK3 signalling.
In Fmr1 KO mice, GSK3 activity is abnormally elevated and lithium treatment was shown to reverse abnormal phenotypes typical of FXS (Min et al., 2009, Neuropharmacol. 56: 463-72; Guo et al., 2012, Hum. Mol. Genet. 21: 681-91). Consistently, we suggest that activation of 5-HT7 receptors, by inhibiting GSK3 activity, might correct the pathological features in Fmr1 KO mice and might be considered as a novel therapeutic strategy for FXS.

Disclosures: L.T. Costa: None. **C.M. Bonaccorso:** None. **S.A. Musumeci:** None. **M.V. Catania:** None. **L. Ciranna:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.14/E49

Topic: B.08. Synaptic Plasticity

Support: NIH grants NS034007

NIH grants NS047384

Department of Defense CDMRP award W81XWH-11-1-0389

Title: Investigations of the role of eIF4E/eIF4G interactions and S6K1 in protein synthesis-dependent hippocampal synaptic plasticity

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[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Ctr. for Neural Sci., [3]New York Univ., New York City, NY

Abstract: Memories are encoded and stored through persistent, activity-dependent changes at individual synapses. Long-term depression (LTD) is an activity-dependent decrease in synaptic efficacy that can be induced in hippocampal area CA1 by pharmacological application of the selective group I metabotropic glutamate receptor (mGluR) agonist 3,5-diyhroxyphenylglycine (DHPG). Current evidence indicates that mGluR-LTD requires new protein synthesis, achieved through activation of mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. The mTORC1 cascade regulates cap-dependent translation by modulating both initiation and elongation phases. Upon activation, mTORC1 induces phosphorylation of 4E-binding protein (4E-BP), thereby releasing the cap-binding translation factor eIF4E so that it can bind to eIF4G, which recruits other initiation factors (eIF4B and eIF4A) to form the initiation complex eIF4F. mTORC1 also activates p70 S6 kinase 1 (S6K1), which in turn phosphorylates eIF4B and the elongation factor kinase eEF2. Phosphorylation of eIF4B promotes the helicase activity of eIF4A and phosphorylation of eEF2 kinase regulates via eEF2 the translocation step of elongation. Thus, mTORC1-dependent activation of 4E-BP and S6K1 is a crucial molecular event that modulates cap-dependent protein synthesis.
Recently, two new compounds termed 4EGI-1 and PF-4708671 has been identified that inhibit the downstream effectors of mTORC1. 4EGI-1 blocks the association eIF4E with eIF4G, thereby disrupting the initiation phase of translation. Whereas, PF-4708671 inhibits the activity of S6K1, thus blocking initiation and elongation phases of translation.
Here, we have investigated the impact of 4EGI-1 and PF-4708671 on hippocmapal mGluR-LTD. We have found that neither 4EGI-1 nor PF-4708671 alone impact hippocampal mGluR-LTD. In contrast, we found that hippocampal slices treated with both 4EGI-1 and PF-4708671 attenuates mGluR-LTD. These results are consistent with the attenuation of mGluR-LTD by rapamycin, which by blocking mTORC1, inhibits both 4E-BP and S6K1. Our findings indicate that concomitant activation of eIF4F and S6K1 are required for mGluR-LTD and that 4EGI-1 and PF-4708671 are effective tools for examining specific translational control mechanisms required for long-lasting synaptic plasticity. Moreover, our findings suggest that 4EGI-1 and PF-4708671 have the potential as therapeutics for cognitive disorders, including autism spectrum disorders, that are associated with enhanced eIF4E/eIF4G interactions and increased S6K activity.

Disclosures: E. Santini: None. **T. Huynh:** None. **S. Koo:** None. **E. Klann:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.15/E50

Topic: B.08. Synaptic Plasticity

Support: NIH-R01 NS065067

Title: Epigenetic regulation of synaptic plasticity and epilepsy in tuberous sclerosis complex

Authors: *T. BASU, K. O'RIORDAN, A. KIRCHNER, B. SCHOENIKE, A. ROOPRA; Univ. of Wisconsin-Madison, Madison, WI

Abstract: Tuberous Sclerosis Complex (TSC) is a genetic disorder that affects 1 in 6,000 people and it is characterized by epilepsy, benign tumor formations throughout the body and brain, and cognitive deficits (Curatolo, P., 2002; Crino, P.B. 2006). TSC is caused by a mutation in either *TSC1* or *TSC2* genes resulting in heightened mammalian target of Rapamycin (mTOR) signaling (van Slegtenhorst, 1997). Mammalian TOR is a protein kinase that regulates activity dependent translation of dendritic proteins required for synaptic plasticity in the hippocampus. Persistent mTOR activation in TSC leads to heightened dendritic protein synthesis and aberrant synaptic plasticity deficits (Tang, S.J., 2002; Hou,L., 2004).

Heterozygous TSC2 mutants, ($TSC2^{+/-}$) exhibit abnormal long term potentiation (LTP) and long term depression (LTD) compared to Wild Type (WT) mice: whereas a 1X Theta Burst Stimulation elicits short term potentiation in WT mice, this stimulation produces LTP in $TSC2^{+/-}$ mice. In contrast to WT mice, adult $TSC2^{+/-}$ mice display rapamycin insensitive metabotropic glutamate receptor (mGluR) mediated LTD. Epileptiform activity in hippocampal slices can be induced through prolonged incubation with the group 1 mGluR agonist, DHPG. Recordings from the CA3 stratum pyramidale region of the hippocampus show that $TSC2^{+/-}$ mice have greater ictal-like activity (burst durations lasting longer than 2 seconds with greater than 2 Hz of intraburst frequency) and more long duration burst activity compared to WT mice.

From a whole genome expression analysis of cortical samples collected from TSC patients and non-TSC patients, we found that the majority of gene expression changes between the two populations can be explained by epigenetic mechanisms. Using drugs that modify chromatin structure through histone deacetylation and demethylation, we find that the aberrant forms of LTP, LTD and bursting phenotypes present in $TSC2^{+/-}$ are ameliorated. Our findings are the first to describe epigenetic mechanisms influencing the synaptic plasticity alterations in TSC. This novel study opens up the possibility of using clinically available epigenetic modifying drugs to treat the cognitive, synaptic plasticity and epilepsy manifest in TSC patients.

Disclosures: T. Basu: None. **A. Roopra:** None. **K. O'Riordan:** None. **A. Kirchner:** None. **B. Schoenike:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.16/E51

Topic: B.08. Synaptic Plasticity

Support: NIH Grant P01 DA 12408

Danish Medical Research Council

Lundbeck Foundation Center for Biomembranes in Nanomedicine

Novo Nordic Foundation

University of Copenhagen BioScaRT Program of Excellence

Title: AMPA receptor pHluorin-GluA2 reports NMDA receptor-induced intracellular acidification in hippocampal neurons

Authors: *M. A. RATHJE[1], H. FANG[2], J. L. BACHMAN[2], U. GETHER[1], R. L. HUGANIR[2], K. L. MADSEN[1];
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Abstract: NMDA receptor activation promotes endocytosis of AMPA receptors, which is an important mechanism underlying LTD (long term synaptic depression). The pH-sensitive GFP variant pHluorin fused to the N-terminus of GluA2 (pH-GluA2) has been used to assay NMDA-mediated AMPA receptor endocytosis and recycling. Here, we demonstrate that in somatodendritic regions of hippocampal neurons a large fraction of the fluorescent signal originates from intracellular pH-GluA2, and that the decline in fluorescence in response to NMDA and AMPA primarily describes an intracellular acidification, which quenches the pHluorin signal from intracellular receptor pools. Neurons expressing an ER-retained mutant of GluA2 (pH-GluA2 ΔC49) displayed a larger response to NMDA than neurons expressing wild type pH-GluA2. A similar NMDA-elicited decline in pHluorin signal was observed by expressing cytosolic pHluorin alone without fusion to GluA2 (cyto-pHluorin). Intracellular acidification in response to NMDA was further confirmed by using the radiometric pH-indicator carboxy-SNARF-1. The NMDA-induced decline was followed by rapid recovery of the fluorescent signal from both cyto-pHuorin and pH-GluA2. The recovery was sodium-dependent and sensitive to Na+/H+-exchanger (NHE) inhibitors. Moreover, recovery was prolonged by shRNA-mediated knock-down (KD) of the GluA2 binding PDZ-domain protein PICK1.

Interestingly, the accelerating effect of PICK1 KD on the fluorescent recovery was eliminated in the presence of the NHE1 inhibitor zoniporide. Our results challenge the applicability of pH-GluA2 for studying AMPA receptor trafficking and suggest a role of PICK1 in regulating intracellular pH via modulation of NHE activity.

Disclosures: M.A. Rathje: None. **U. Gether:** None. **R.L. Huganir:** None. **K.L. Madsen:** None. **J.L. Bachman:** None. **H. Fang:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.17/E52

Topic: B.08. Synaptic Plasticity

Support: CONACyT Grant 154131

CONACyT Grant 98004

DGAPA-UNAM Grant IN206010

DGAPA-UNAM Grant IN205610

IMPULSA 03-UNAM to EG and JB

Title: Striatopallidal long-term synaptic plasticity

Authors: *R. HERNÁNDEZ, SR, M. A. ARIAS-GARCÍA, J. E. PEREZ-ORTEGA, E. GALARRAGA, J. BARGAS;
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Abstract: Long-term synaptic plasticity (LTSP) has been proposed as a mechanism for information storage at both excitatory and inhibitory synapses. It is posited as the cellular/molecular basis of memory. The basal ganglia are involved in the storage of procedural memories. However, current research of LTSP in the basal ganglia has almost completely been restricted to the corticostriatal synapse. Nonetheless, it would be strange that all memory process in these subcortical nuclei depend on a single synapse. Thus, our laboratory has demonstrated that complex LTSP phenomena are present in striatonigral inhibitory synapses (direct pathway of the basal ganglia). Here, we now show, by using whole cell patch clamp recordings from pallidal neurons (GPe) in rat brain slices, that the striatopallidal inhibitory synapse (indirect pathway of the basal ganglia) exhibits LTSP in the form of long term synaptic depression (LTD).

Metabotropic receptors such as muscarinic and glutamatergic are necessary to induce this phenomenon via the production of endocannabinoids synthesis. However, once induced, the mechanism that stabilize and sustain LTD does not longer depend on endocannabinoids.

Disclosures: R. Hernández: None. **M.A. Arias-garcía:** None. **J.E. Perez-ortega:** None. **E. Galarraga:** None. **J. Bargas:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.18/E53

Topic: B.08. Synaptic Plasticity

Support: NIH R01-NS044421-08 to PKS

NIH F31-NS080605-01 to KRG

Title: N-methyl-d-aspartate receptor (nmdar)-activated kinases in presynaptic long-term depression (ltd)

Authors: *K. R. GOPAUL[1], X.-L. ZHANG[2], P. STANTON[3];
[1]New York Med. Col., New York, NY; [2]Cell Biol. & Anat., New York Med. Col., Valhalla, NY; [3]Cell Biol. & Anat., New York Med. Col., New York, NY

Abstract: *N*-methyl-D-aspartate receptors (NMDAR) and metabotropic glutamate receptors (mGluR) have independently been shown to play important roles in induction of long-term depression (LTD) of presynaptic transmitter release at a variety of hippocampal synapses. Postsynaptic NMDAR activation leads to generation of the intercellular messenger nitric oxide, which triggers presynaptic production of cyclic guanosine monophosphate (cGMP) that transiently depresses transmitter release. Activation of presynaptic group II mGluR, a G protein-coupled receptor (GPCR), releases Gα and Gβγ subunits that play distinct roles in the reduction of vesicle release. We have shown that increased [cGMP] evoked by NMDAR activation, combined with inhibition of adenylate cyclase (AC) by an inhibitory $G\alpha_{i,}$, induces LTD. The other G protein released after GPCR activation, Gβγ, regulates transient depression of neurotransmitter release by interacting with the C-terminus of the SNARE (Soluble N-ethylmaleimide-Sensitive Factor Attachment Protein Receptor) protein, Synaptosomal Associated Protein 25kD (SNAP-25). We hypothesize that three events, elevated [cGMP], inhibition of PKA by $G\alpha_{i}$, and binding of Gβγ to the C-terminus of SNAP-25 are all necessary for the induction of LTD of vesicular neurotransmitter release. We monitored changes in release

mechanisms from distinct readily-releasable and reserve presynaptic vesicle pools, using electrophysiological recordings in the hippocampus of rodent brain slices and imaging fluorescent membrane dyes destaining via two-photon microscopy. By selectively inhibiting enzymes in the NMDAR or mGluRII biochemical pathways, we used the above techniques to determine the shared regulatory component of neurotransmitter release from Schaffer collateral presynaptic terminals in the CA1 region of hippocampal slices. Blockade of CaMKII activity with the selective inhibitors KN-62 (2 µM) or KN-93 (2 µM) partially inhibited LTD of both synaptic transmission and FM1-43 release from the sucrose-loaded readily-releasable vesicle pool elicited by bath application of NMDA (20 µM), but not mGluRII-LTD elicited by the mGluRII agonist DCG-IV (25 µM). These data suggest that a presynaptic component of NMDAR-dependent LTD depends on CaMKII activity, probably in the presynaptic terminal.

Disclosures: K.R. Gopaul: None. **X. Zhang:** None. **P. Stanton:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.19/E54

Topic: B.08. Synaptic Plasticity

Support: NIH Grant 1R21AG031467

NIH Grant 5R01AG028271

NIH Grant 1R01AG41944

Title: Aging-associated immune dysregulation drives a shift in the direction of hippocampal synaptic plasticity and the ratio of proBDNF vs. mBDNF

Authors: *G. P. CORTESE, R. M. BARRIENTOS, S. F. MAIER, S. L. PATTERSON; Psychology & Neurosci., Univ. of Colorado, Boulder, BOULDER, CO

Abstract: Older individuals often experience precipitous declines in cognitive function after events (Ex. surgery, infection, or injury) that trigger activation of the peripheral immune system. Aging sensitizes the hippocampal inflammatory response to peripheral infection, increasing the

size and duration of the resulting spike in interleukin-1beta (IL-1β). We have previously demonstrated that in aging (24 month), but not in young (3 month) F344xBN rats, a peripheral immune challenge (i.p. injection of live E. coli) triggers an exaggerated elevation in hippocampal IL-1β, which in turn disrupts forms of long-term memory and synaptic plasticity known to be BDNF-dependent (Barrientos et al. 2006; Chapman et al., 2010).
Hippocampal memory processes are thought to involve shifts in the balance of LTP and LTD (long-term potentiation and depression of excitatory synaptic transmission). Numerous studies have shown that disruptions in hippocampal LTP (Barnes and McNaughton 1985), and enhancements of hippocampal LTD (Massey & Bashir 2007) are strongly correlated with memory impairments. In addition, shifts in the direction of hippocampal synaptic plasticity have been reported in rodent models of neurodegenerative diseases associated with memory loss (Li et al., 2009).
Interestingly, BDNF modulates both LTP and LTD. BDNF is synthesized as a precursor protein (proBDNF), and cleaved to produce the mature BDNF protein isoform (mBDNF). Pro-BDNF binds preferentially to the pan-neurotrophin receptor p75NTR, activates apoptosis-related signaling pathways, and facilitates long-term depression (LTD) in the hippocampus. In contrast, mBDNF binds to TrkB receptors, promotes cell survival, and is required for some forms of long-term potentiation (LTP).
We have previously found that mBDNF, but not pro-, is significantly reduced in hippocampal synaptoneurosomes prepared from aged animals following an infection (Cortese et al., 2011) - an observation consistent with reduced theta burst L-LTP at Schaffer collateral-CA1 synapses in these animals (Chapman et al., 2010). Here, we report that the IL-1β -driven shift in the ratio between proBDNF and mature BDNF is also associated with enhanced LTD, and evidence of increased proBDNF - p75 receptor-ligand interactions. This work examines the functional role of endogenous BDNF protein isoforms in memory-related plasticity processes. It may also provide novel insights into the early stages of synaptic failure in a variety of disorders associated with dysregulated brain inflammatory responses (Ex. post-operative cognitive dysfunction, autoimmune diseases, depression, PTSD and some neurodegenerative disorders).

Disclosures: G.P. Cortese: None. **R.M. Barrientos:** None. **S.F. Maier:** None. **S.L. Patterson:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.20/F1

Topic: B.08. Synaptic Plasticity

Support: The Lundbeck Foundation

Title: Impairment of both NMDAR-dependent and mGluR-dependent long-term depression (LTD) in the hippocampus of a sortilin related receptor (SORCS3) deficient mouse model

Authors: *G. B. CHRISTIANSEN[1], T. BREIDERHOFF[3], A. NYKJAER[2], K. JENSEN[1,2], T. WILLNOW[3], M. M. HOLM[1];
[1]Dept. of Biomedicine, [2]Lundbeck Fndn. Res. Ctr. MIND, Dept. of Biomedicine, Aarhus Univ., Aarhus C, Denmark; [3]Max-Delbrueck-Centrum (MDC), Berlin, Germany

Abstract: SORCS3 is a member of the VPS10P-domain receptor family also known as the sortilins. So far, this receptor has been the most anonymous member whereas other members of the family are believed to be involved in synaptic plasticity and different disorders of the CNS. *In situ* hybridization analyses have previously showed that SORCS3 is strongly expressed in the CA1 of the hippocampus (Hermey et al., 2004, *J. of Neurochem.*) and consequently, we initiated electrophysiological analysis in this area on a SORCS3 deficient mouse model to investigate the role of this receptor in synaptic plasticity.
Extracellular field recordings of Schaffer collateral-pyramidal cell synapses in the CA1 area of the hippocampus were performed in an interface recording chamber. 400 µm thick coronal slices were prepared from age-matched wild-type (WT) and knockout (KO) animals. Young mice (P17-P21) were included in the long-term depression (LTD) analysis, and mature mice (P30-P60) were included in the long-term potentiation (LTP) analysis and paired-pulse recordings. NMDAR-dependent LTD was induced by a 20 min 1 Hz protocol, and the mGluR-dependent LTD was induced by an 18 min 1 Hz with paired-pulse (interstimulus interval at 50 ms) protocol. LTP was induced by a 2 x 100 Hz protocol. The slope of the field excitatory postsynaptic potential (fEPSP) was normalized to a 20 min baseline.
These data revealed that the SORCS3 protein has an essential role in synaptic plasticity. Both the NMDAR-dependent (WT: 83.6% ± 4.2%; KO: 99.2% ± 4.9%, $p < 0.05$) and mGluR-dependent LTD (WT: 78.3% ± 8.3%; KO: 98.1% ± 4.4%, $p < 0.05$) was impaired in the SORCS3 deficient mice. Early LTP was normal (WT: 158% ± 11.0%; KO: 143% ± 8.7%, $p > 0.05$) and paired-pulse analysis using interstimulus intervals of 25-300 ms revealed no obvious presynaptic defects in SORCS3 knockout mice ($p > 0.05$). Application of the $GABA_B$ receptor agonist baclofen increased the paired-pulse ratio equally in WT and KO mice ($p > 0.05$) from 180% to 206% in WT mice and from 184% to 200% in KO mice (interstimulus interval at 50 ms).
Based on these results, we conclude that the SORCS3 receptor is likely to play an essential postsynaptic role in NMDAR-dependent and mGluR-dependent LTD, probably through interactions with key proteins responsible for the internalization and/or retention of AMPA receptors during LTD. Based on the fEPSP profile during the LTD analysis, the retention of the AMPA receptors within intracellular compartments appears to be affected the most by the absence of the SORCS3 receptor.

Disclosures: G.B. Christiansen: None. **T. Breiderhoff:** None. **A. Nykjaer:** None. **K. Jensen:** None. **T. Willnow:** None. **M.M. Holm:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.21/F2

Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH071666

NIH Grant EY02858

the G. Harold and Leila Y. Mathers Charitable Foundation

Title: Neuronal expression of H2-Db is sufficient for synaptic pruning and regulation of AMPA receptors

Authors: *H. LEE, B. K. BROTT, C. J. SHATZ;
Depts. Biology, Neurobiology, and Bio-X, James H. Clark Ctr., Stanford Univ., STANFORD, CA

Abstract: Major Histocompatibility Class I (MHCI) proteins, discovered in an unbiased screen for genes regulated by activity, are candidate key molecules for mediation of activity-dependent synapse remodeling. In mice lacking two MHCIs, H2-Kb and H2-Db (KbDb-/-), despite intact retinal activity and basal synaptic transmission, the developmentally regulated decrease in functional convergence of retinal ganglion cell inputs to LGN neurons fails, and eye-specific layers do not form. Neuronal expression of just H2-Db in KbDb-/- mice rescues both synapse pruning and eye specific segregation, despite a compromised immune system, underscoring a role for these molecules in neurons.

To study cellular mechanisms underlying the failure of pruning, whole cell recordings of EPSCs were made from LGN neurons in KbDb-/- vs WT mice. Prolonged AMPA currents at -70mV in KbDb-/- slices suggested the presence of Ca2+ permeable AMPA receptors. Consistently, decay time of EPSCs was significantly increased in KbDb-/- (WT: 13±4 ms; KbDb-/-: 55±15 ms, $p < 0.05$). To examine if there are changes in Ca2+ permeability, we analyzed subunit composition in AMPARs. Slightly increased GluA1 subunit protein levels but no changes in the GluA2 subunit are detected in KbDb-/- thalamus, suggesting an altered GluA1/2 ratio. To study the link between AMPAR subunit composition and H2-Db further, cortical neurons were studied in vitro. Using biotinylation and H2-Db/Kb-specific antibodies, H2-Db protein was detected on the cell

surface. Next, layer 2/3 pyramidal neurons were labeled by in-utero electroporation and then grown in vitro. Whole cell recordings from these neurons show that amplitude and frequency of mEPSCs (Vh -70 mV) are significantly increased in KbDb-/- (Amplitude:WT: 18.5 pA± 1.6; KbDb-/-: 23.7pA± 1.4, $p < 0.05$; Frequency: WT:1.9Hz± 0.9, n=10; KbDb-/-: 5.5 Hz ± 1.0, n=9, $p < 0.05$). GluA1 protein levels are also significantly increased ($p<0.03$) in KbDb-/- cortical neuronal cultures, with no significant changes in GluA2, yielding a higher GluA1/2 ratio. Thus, the subunit composition of AMPA receptors is altered in favor of Ca2+ permeability through AMPA receptors in KbDb-/-. These observations suggest that H2-Db regulates GluA1 levels in AMPARs, leading to altered Ca2+ permeability. The increase in Ca2+ influx through AMPARs in KbDb-/- could bias synaptic learning rules at retinogeniculate synapses away from LTD and towards LTP, thereby preventing synaptic weakening leading to synapse removal and pruning.

Disclosures: H. Lee: None. **B.K. Brott:** None. **C.J. Shatz:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.22/F3

Topic: B.08. Synaptic Plasticity

Support: SFFR F46.2/001

NASU Biotechnology

NASU 0112U001475

Title: Hippocalcin signaling in hippocampal LTD

Authors: *P. V. BELAN[1,2], A. DOVGAN[1], N. KONONENKO[1], V. CHERKAS[1], T. TSUGORKA[1], L. HAYNES[3], R. D. BURGOYNE[3];
[1]Bogomoletz Inst. Physiol, Kiev, Ukraine; [2]Key State Lab. of Mol. and Cell. Biol., Kiev, Ukraine; [3]Univercity of Liverpool, Liverpool, United Kingdom

Abstract: It has been previously shown that neuronal Ca2+ sensor protein, hippocalcin (HPCA), functions as a Ca2+ sensor in NMDAR-dependent LTD in the hippocampus. However, particular biophysical mechanisms of HPCA signaling during expression of LTD have not been studied yet. Here we show that simultaneous postsynaptic depolarization and low frequency presynaptic stimulation of cultured hippocampal neurons resulted in HPCA translocation to the plasma membrane associated with LTD of AMPAR-mediated excitatory postsynaptic currents. The

translocation was observed in 1-3 um sites on the plasma membrane located in both dendritic spines and shaft. HPCA was gradually (10-100 s) accumulated in the translocation sites integrating presynaptic neuronal activity. Translocated HPCA was being inserted into the plasma membrane for a prolonged period of time (decay constant 93±36 s) after termination of LTD expression protocol. This decay was significantly longer compared with a decay of HPCA translocation induced in the same set of sites after high frequency stimulation (6.2±3.0 s). Besides, sets of sites revealing translocation to low and high frequency presynaptic stimulation were not overlapped indicating that HPCA may decode different presynaptic inputs into specific spatio-temporal patterns of translocation in the plasma membrane of dendritic tree. Neither postsynaptic depolarization nor presynaptic stimulation alone did not produce HPCA translocation and only their association resulted in significant and substantial translocation. Calcium imaging in translocation sites demonstrated a moderate steady state increase of [Ca2+]i in a response to the postsynaptic depolarization and a fast transient responses to presynaptic stimulation. HPCA effectively filtered out these types of [Ca2+]i changes and translocated to the plasma membrane sites only in case when pre- and postsynaptic activity was properly associated in time. Thus, we conclude that biophysical properties of HPCA are perfectly suited to nonlinearly and associatively decode neuronal activity occurring during expression of LTD leading to HPCA insertion into the plasma membrane sites where it may initiate AMPAR endocytosis.

Disclosures: P.V. Belan: None. **A. Dovgan:** None. **N. Kononenko:** None. **V. Cherkas:** None. **T. Tsugorka:** None. **L. Haynes:** None. **R.D. Burgoyne:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.23/F4

Topic: B.08. Synaptic Plasticity

Support: DICBR

Title: Roles for presynaptic N- and P/Q-type calcium channels in plasticity of cortical afferents in the dorsolateral striatum

Authors: *D. A. KUPFERSCHMIDT, D. M. LOVINGER;
Synaptic Pharmacol., NIAAA / NIH, Rockville, MD

Abstract: The dorsal striatum is essential for the formation of habits and motor skills, and these forms of learning are hypothesized to involve plasticity at striatal synapses. Cortical inputs to the

dorsolateral striatum (DLS) exhibit various forms of plasticity that manifest as short- or long-term depression (STD, LTD) of glutamate release. Despite the presynaptic locus of expression of corticostriatal STD and LTD, their presynaptic mechanisms remain largely unexplored. We developed a novel approach to detecting presynaptic calcium (Ca2+) influx in cortical terminals in the DLS, and used it to probe the involvement of presynaptic Ca2+ in corticostriatal plasticity. Following Cre-dependent viral expression of the genetically encoded Ca2+ indicator GCaMP6 in motor cortex projection neurons of Emx1-Cre mice, we used fluorescence photometry to record, in slice, electrically evoked presynaptic Ca2+ transients (preCaTs) within the DLS. Tetrodotoxin-sensitive Na+ channels, cadmium-sensitive voltage-gated Ca2+ channels, and extracellular Ca2+ were required to evoke preCaTs. Application of the selective N- and P/Q-type Ca2+ channel blockers, w-conotoxin GVIA and w-agatoxin IVA, during preCaT recording revealed that N-type channels preferentially contribute to preCaTs in motor cortex projections to the DLS. Likewise, N-type channels appear to have a larger role in synaptic transmission evoked by stimulation of glutamatergic afferents in the DLS. Application of the GABA-B agonist, baclofen, and the mGluR2/3 agonist, LY379268, induced STD of both preCaTs and striatal field potentials. Studies are underway to assess the role of presynaptic Ca2+ influx in endocannabinoid- and serotonin-mediated corticostriatal LTD. These findings provide new insight into the presynaptic mechanisms of corticostriatal plasticity that may contribute to striatal-dependent learning.

Disclosures: D.A. Kupferschmidt: None. **D.M. Lovinger:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.24/F5

Topic: B.08. Synaptic Plasticity

Title: Nitric oxide in striatal synaptic plasticity

Authors: *I. RAFALOVICH, J. PLOTKIN, A. MELENDEZ, D. J. SURMEIER;
Physiol., Northwestern Univ., Chicago, IL

Abstract: Disruption in striatal synaptic plasticity contributes to many basal ganglia associated disorders including Parkinson’s and Huntington's disease. Nictric oxide (NO) signaling machinery is abundant in the striatum, however, its role in striatal synaptic transmission is unclear. Using whole-cell brain-slice electrophysiology we observed that NO signaling induced long-term depression (LTD) at cortico and thalamostriatal synapses. This effect was post-

synaptic, dependent on protein kinase G, and augmented by phosphodiesterase inhibition. In addition, NO signaling occluded the conventional, mGluR - dependent LTD through inhibition of L-type calcium currents. These results provide insight into the regulation of corticostriatal synaptic strength with implications for physiological control of motor function in health and disease.

Disclosures: I. Rafalovich: None. **J. Plotkin:** None. **A. Melendez:** None. **D.J. Surmeier:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.25/F6

Topic: B.08. Synaptic Plasticity

Support: BBSRC

MRC

Patrick Wild Centre

Title: Convergence of synaptic pathophysiology in the hippocampus of the Syngap+/- and Fmr1-/y mice

Authors: *S. BARNES[1], A. D. JACKSON[1], E. M. OSTERWEIL[2], N. KOMIYAMA[1], S. G. N. GRANT[1], M. F. BEAR[2], P. C. KIND[1], D. J. A. WYLLIE[1];
[1]Ctr. of Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Intellectual disabilities (IDs) and autism spectrum disorders (ASDs) are genetically heterogeneous disorders that display a high degree of co-occurrence. Despite their genetic heterogeneity, there is emerging evidence that there is convergence on common neuropathological axes, both in terms of the core cellular processes and the biochemical pathways being affected. One such axis is defined by metabotropic glutamate receptor (mGluR)-dependent protein synthesis, and modulators of this process prevent and/or reverse many of the phenotypes associated with mouse models of Fragile X Syndrome (FXS) and Tuberous Sclerosis. ERK-MAPK is a key regulator of mGluR-dependent protein synthesis and a recent screen of ID patients has revealed heterozygous *de novo* mutations in *SYNGAP1*, a negative regulator of the Ras/Erk pathway, in approximately 4% of individuals. We sought to determine

whether mice with heterozygous null mutations in *Syngap1* show similar core cellular deficits to those found in the mouse model of FXS.
One of the prominent phenotypes reported in the *Fmr1*$^{-/y}$ mouse is that a form of hippocampal long-term depression (LTD) mediated by the activation of Group 1 (Gp1) mGluRs is enhanced and independent of new protein synthesis. We examined mGluR-LTD at Schaffer collateral/commissural inputs to CA1 pyramidal neurones in hippocampal slices obtained from *Syngap*$^{+/–}$ mice. Extracellular field recordings reveal that acute application of the Gp1 mGluR agonist dihydroxyphenylglycine (DHPG; 50 µM) induces a form of mGluR-LTD that is of greater magnitude in *Syngap*$^{+/–}$ mice relative to wild-type (WT) littermate controls (57 ± 7 %, *Syngap*$^{+/–}$, n = 14 versus 75 ± 3 %, WT, n = 19). Furthermore mGluR-LTD in *Syngap*$^{+/–}$ mice is insensitive to protein synthesis inhibitors (unpaired t-test $P<0.05$, n = 15). In addition, we find basal levels of protein synthesis to be elevated in hippocampal slices from *Syngap*$^{+/–}$ mice relative to their WT counterparts (145 ± 15 %, *Syngap*$^{+/–}$ versus 100 ± 4 %, WT n = 12).The comparable neuropathophysiology we observe between *Syngap*$^{+/–}$ and *Fmr1*$^{-/y}$ mice suggests that SynGAP and FMRP may converge on similar biochemical pathways raising the intriguing possibility that therapeutic strategies used in the treatment of FXS may also be of benefit for individuals with ID caused by mutations in *SYNGAP1*.

Disclosures: S. Barnes: None. **A.D. Jackson:** None. **E.M. Osterweil:** None. **N. Komiyama:** None. **S.G.N. Grant:** None. **M.F. Bear:** None. **P.C. Kind:** None. **D.J.A. Wyllie:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.26/F7

Topic: B.08. Synaptic Plasticity

Support: IBS grant/CA1202

Title: LTD-inducing stimuli promote cleavage of the synaptic adhesion molecule NGL-3 through NMDA receptors, matrix metalloproteinases, and presenilin/γ-secretase

Authors: *H. LEE, V[1,2], E.-J. LEE[3], Y. SONG[3], E. KIM[3];
[1]Biol. Sci., Inst. of Basic Sci. (IBS), Daejeon, Korea, Republic of; [2]Korea Advanced Inst. of Sci. and Technology(KAIST), Daejeon, Korea, Republic of; [3]Biol. Sci., Inst. of Basic Sci. (IBS),Korea Advanced institute of Sci. and Technology(KAIST), Daejeon, Korea, Republic of

Abstract: Long-term depression (LTD) reduces the functional strength of excitatory synapses through mechanisms that include the removal of AMPA glutamate receptors from the

postsynaptic membrane. LTD induction is also known to result in structural changes at excitatory synapses, including the shrinkage of dendritic spines. Synaptic adhesion molecules are thought to contribute to the development, function, and plasticity of neuronal synapses largely through their trans-synaptic adhesions. However, little is known about how synaptic adhesion molecules are altered during LTD. We report here that NGL-3 (netrin-G ligand-3), a postsynaptic adhesion molecule that trans-synaptically interacts with the LAR family of receptor tyrosine phosphatases and intracellularly with the postsynaptic scaffolding protein PSD-95, undergoes a proteolytic cleavage process. NGL-3 cleavage is induced by NMDA treatment in cultured neurons and low frequency stimulation in brain slices and requires the activities of NMDA glutamate receptors, matrix metalloproteinases (MMPs), and presenilin/gamma-secretase. These results suggest that NGL-3 is a novel substrate of MMPs and gamma-secretase and that NGL-3 cleavage may regulate synaptic adhesion during LTD.

Disclosures: H. Lee: None. **E. Lee:** None. **Y. Song:** None. **E. Kim:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.27/F8

Topic: B.08. Synaptic Plasticity

Support: KAKENHI23590232

Title: Phldb2 regulates the maturation of dendritic spines and AMPA receptor endocytosis during long-term depression

Authors: *M.-J. XIE[1,2], H. YAGI[1], T. IGUCHI[1,2,3], Y. OKA[1,2,3], K. KURODA[1,2], M. YUZAKI[4], S. MATSUDA[4], T. SHIRAO[5], Y. ISHIKAWA[6], M. SATO[1,2,3,7];
[1]Morphological and Physiological Sci., Univ. of Fukui, Fac. of Med. Sci., Fukui,, Japan; [2]Res. and Educ. Program for Life Sci., [3]Res. Ctr. for Child Mental Develop., Univ. of Fukui, Fukui, Japan; [4]Dept. of Neurophysiol. Sch. of Med., Keio Univ., Tokyo, Japan; [5]Dept. of Neurobio. and Behavior, Gunma Univ. Grad. Sch. of Med., Maebashi, Japan; [6]Lab. of Functional Neurosci., Grad. Sch. of Biol. Sciences, Nara Inst. of Sci. and Technol., Nara, Japan; [7]Dept. of Anat. and Neurosci., Osaka Univ. Grad. Sch. of Med., Osaka, Japan

Abstract: Synapse function and plasticity depend on the morphology of dendritic spine. Dendritic filopodium is highly dynamic structure, known as a premature form of the dendritic spine. Here, we report that phldb2 (pleckstrin homology-like domain, family B, member 2), one binding partner for a well-known actin-cross-linking protein Filamin A, works as a positive

regulator of spine maturation. We generated Phldb2-knockout mice and found that a proportion of immature spines (filopodia and thin spines) increased in the hippocampus in vivo, which is consistent with our previous observations with Phldb2 knocked-down cultured hippocampal neurons. Next, we asked whether or not Phldb2 is involved in synaptic plasticity. We observed that NMDA-induced AMPA receptor endocytosis and low-frequency stimulation-induced long-term depression were blocked in hippocampal neurons of the Phldb2-knockout mice. Therefore, it is likely that Phldb2 plays an important role for the maturation of dendritic spines and for the synaptic plasticity.

Disclosures: M. Xie: None. **H. Yagi:** None. **T. Iguchi:** None. **Y. Oka:** None. **K. Kuroda:** None. **M. Yuzaki:** None. **S. Matsuda:** None. **T. Shirao:** None. **Y. Ishikawa:** None. **M. Sato:** None.

Poster

518. **Long-Term Depression (LTD)**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 518.28/F9

Topic: B.08. Synaptic Plasticity

Support: HFSP

BBSRC

MRC

Wellcome trust

Ministerio de Ciencia e Innovación

Title: Presynaptic NMDA receptor-dependent self-depression at developing neocortical synapses

Authors: *A. RODRIGUEZ-MORENO[1,2,3], A. GONZÁLEZ-RUEDA[3], A. BANERJEE[2], A. L. UPTON[2], M. T. CRAIG[2], O. PAULSEN[3,2];
[1]Univ. Pablo de Olavide (Edificio 21), Seville, Spain; [2]Univ. of Oxford, Oxford, United Kingdom; [3]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: A central tenet of most theories of synaptic modification during cortical development is that correlated activity drives plasticity in synaptically-connected neurons. In contrast, using sensory-evoked activity patterns recorded from the developing mouse cortex in vivo, we uncovered a synaptic learning rule that relies solely on the presynaptic neuron. A burst of three

presynaptic spikes at a frequency of at least 50 Hz followed, within a restricted time window of 50-200 ms, by a single presynaptic spike induced robust long-term depression (LTD) at developing layer 4-to-layer 2/3 synapses. This presynaptic spike pattern-dependent LTD (p-LTD) could be induced by individual presynaptic layer 4 cells in paired recordings of synaptically connected neurons, required presynaptic NMDA receptors and calcineurin, as it was blocked by loading MK801 or FK506 into the presynaptic neuron via the patch pipette, and was expressed presynaptically as indicated by fluctuation analysis. Spike timing-dependent LTD (t-LTD) and p-LTD converge mechanistically on NMDA receptors, as they occluded each other, but in contrast to t-LTD, p-LTD did not require mGluRs, postsynaptic Ca2+-dependent processes, CB1 receptors or astroglial signalling. A form of p-LTD was also observed at horizontal synapses between L2/3 neurons. This spike pattern-dependent learning rule complements timing-based rules and is likely to play a role in the pruning of synaptic input during cortical development.

Disclosures: A. Rodriguez-Moreno: None. **A. González-Rueda:** None. **A. Banerjee:** None. **A.L. Upton:** None. **M.T. Craig:** None. **O. Paulsen:** None.**Poster**

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.01/F10

Topic: B.08. Synaptic Plasticity

Support: NIH/NIMH grant RO1MH087631

Title: Heterosynaptic plasticity of excitatory inputs to inhibitory neurons in rat visual cortex *In vitro*

Authors: *V. ILIN[1], M. ROSCHIN[1,2], M. CHISTYAKOVA[1], M. VOLGUSHEV[1];
[1]Psychology, Univ. of Connecticut, Storrs, CT; [2]Inst. of Higher Nervous Activity and Neurophysiol., Moscow, Russian Federation

Abstract: Long-term changes are typically studied at synapses that were activated during plasticity induction protocol - homosynaptic plasticity. However, Hebbian learning rules introduce a positive feedback on synaptic weight changes, making them prone to runaway dynamics. In our prior work we have described a form of plasticity in neocortical pyramidal neurons that can be induced by intracellular tetanization - bursts of postsynaptic action potentials, without presynaptic stimulation. This form of heterosynaptic plasticity (plasticity at synapses that were not active during the induction) can effectively prevent runaway dynamics of synaptic weights and neuronal firing.

Here we ask: (i) whether heterosynaptic plasticity can be induced by intracellular tetanization at excitatory inputs to inhibitory neurons in the neocortex, and (ii) whether heterosynaptic plasticity in inhibitory cells has different properties from those in excitatory neurons. We made whole-cell recordings from inhibitory neurons in slices from rat visual cortex. Excitatory postsynaptic potentials were evoked in the recorded cells by paired pulses applied to bipolar extracellular electrodes. Intracellular tetanization (3 trains of 10 bursts of 5 spikes at 100 Hz) induced long-term potentiation in 41 of 120 (34%) inputs to inhibitory neurons, long-term depression in 37 (31%) inputs, and did not change responses in 42 (35%) inputs. These plastic changes differed from those induced by intracellular tetanization in pyramidal neurons in the following respects. First, the direction of plastic changes in inhibitory neurons did not depend on initial paired-pulse ratio, as it did in pyramidal neurons. Second, the direction of plasticity in inhibitory neurons was correlated with the slope of EPSP: responses with steeper slopes had higher probability to be potentiated, while responses with slower slopes expressed depression more often. This correlation indicates possible distance-dependence of predispositions for potentiation and depression. Third, the magnitude of potentiation in inhibitory neurons (143+25%) was smaller than in excitatory neurons (171+56%, $p<0.001$). Similar to excitatory cells, long term plasticity in inhibitory neurons was associated with changes of release indices. EPSP amplitude changes were negatively correlated with changes of paired-pulse ratio, and positively correlated with changes of inversed coefficient of variation, indicating involvement of presynaptic mechanisms. Since presynaptic fibers were not activated during our induction protocol, presynaptic changes imply involvement of retrograde signaling.

Disclosures: V. Ilin: None. **M. Chistyakova:** None. **M. Volgushev:** None. **M. Roschin:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.02/F11

Topic: B.08. Synaptic Plasticity

Title: External glucose concentration has influences on the spontaneous electrical activity in dissociated neuronal network

Authors: *W. MINOSHIMA[1], H. ITO[2], S. KUDOH[3];
[1]Kudoh Lab. Dep. of Human Sys. Int., Japan/Graduate Sch. of Sci. and Tech., Kwansei Gakuin Univ, S. N. Kudoh's Lab., Sanda/Hyogo, Japan; [2]Kwansei Gakuin University, Sch. of Sci. and Tech., Kwansei Gakuin Univ, S. N. Kudoh's Lab,, Sanda/Hyogo, Japan; [3]Kwansei Gakuin

University, Sch. of Sci. and Tech., Kwansei Gakuin Univ, S. N. Kudoh's Lab,, Sanda/ Hyogo, Japan

Abstract: Cultured rat hippocampal neuronal network on a multi electrode array (MEA) dish is a useful model for analyzing electrical network dynamics. Neurons autonomously form a complex network on a dish with 64 planar microelectrodes array. Autonomous electrical activity without evoking stimulus was recorded from the electrodes. The autonomous activity is generated by mutual interaction between neurons and it reflects internal states of neuronal network. It is plausible that the autonomous activity is influenced by energy intake. We elucidated the relationship between autonomous electrical activity and external glucose concentrations. The number of electrical spikes in autonomous activity increases depending on external glucose concentration. The number of spikes is the most at 15 mM glucose concentration and then decreased when the concentration was more than 20 mM. The glucose concentration for the most frequent activity was near the concentration in the culture medium, suggesting that the neurons were adapted to that concentration. In addition, the decrease of spikes at high glucose concentration was not influenced by the blockade of inhibitory synaptic inputs. These results suggest that autonomous activity in cultured neuronal networks depends on the amount of an external energy source.

Disclosures: W. Minoshima: Other; Suguru N. Kudoh. **H. Ito:** Other; Suguru N. Kudoh. **S. Kudoh:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relation.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.03/F12

Topic: B.08. Synaptic Plasticity

Support: NIH/NIMH grant RO1MH087631

Title: Heterosynaptic plasticity and synaptic competition

Authors: *J.-Y. CHEN[1], C. LEE[2], M. CHISTIAKOVA[2], M. VOLGUSHEV[2], M. BAZHENOV[1];

[1]Dept. of Cell Biol. and Neurosci., Univ. of California, Riverside, Riverside, CA; [2]Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Spike timing dependent synaptic plasticity (STDP) is experimentally well-characterized form of plasticity that modifies synaptic weights depending on the relative timing of presynaptic input and postsynaptic spikes. This formal rule is broadly used in computational models of learning and developmental processes. However, STDP and other conventional Hebbian-type plasticity rules impose a positive feedback on synaptic changes, and are prone to produce runaway dynamics of synaptic weights and neuronal activity. Nevertheless, learning of complex associations for both declarative and procedural memories requires a broad distribution of synaptic weights. To achieve this and to keep system susceptible for new learning, stabilization mechanisms that prevent synapses from runaway potentiation or depression and associated over-excitability or complete silencing of neurons are necessary. In our prior work we described a form of heterosynaptic plasticity in vitro that can be induced by intracellular tetanization - a purely postsynaptic protocol without presynaptic stimulation. We implemented this form of heterosynaptic plasticity in computer model and demonstrated that it can serve as a normalizing mechanism and prevent runaway synaptic dynamics over a broad range of STDP rules. Here, we explored whether stabilizing effect of heterosynaptic plasticity still leaves room for synaptic competition. Synaptic inputs to a model neuron have been segregated into groups, which either expressed different degree of correlation between firing of presynaptic neurons, or fired at different rates. In the STDP-only model, synapses receiving highly correlated (or high firing rate) inputs were rapidly potentiated and their weights saturated at the maximal value. Synapses receiving weakly correlated (or low firing rate) inputs expressed little plasticity, and their distribution essentially did not change. In the model with both STDP and heterosynaptic plasticity, final synaptic weights of synapses from different groups formed compact and clearly separated distributions. In contrast to the STDP-only model, none of the synapses expressed runaway dynamics, but all synaptic weights remained within the operation range. We conclude that although heterosynaptic plasticity effectively counteracts runaway dynamics of synaptic weights, it does not preclude activity-dependent plasticity. Synapses from presynaptic neurons that fire together (or at higher rate) acquire higher weights than synapses from presynaptic neurons that exhibit low-correlated (or low rate) firing.

Disclosures: J. Chen: None. **C. Lee:** None. **M. Chistiakova:** None. **M. Volgushev:** None. **M. Bazhenov:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.04/F13

Topic: B.08. Synaptic Plasticity

Support: MRC Studentship

Title: Ripples enable replay-induced synaptic plasticity in the hippocampus

Authors: *J. SADOWSKI, J. R. MELLOR, M. W. JONES;
Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: Mounting evidence suggests that hippocampal sharp wave/ripple oscillations play an important role in memory consolidation. Ripples occur primarily during off-line quiescent states and are preferentially associated with replay of sequential activity associated with recent behavioural episodes. It is not known how ripple-associated replay facilitates memory consolidation, but synaptic plasticity is likely to play a pivotal role. Here we test whether replay activity recorded from hippocampal place cells in vivo is capable of inducing synaptic plasticity in hippocampal slices.

Tetrodes were used to record multiple single unit and local field potential (LFP) simultaneously from CA3 and CA1 of the dorsal hippocampus during alternating periods of track running and quiescence in 3 adult male Wistar rats (10-12 weeks old); place field, cross-correlation and spectral analyses were used to examine experience-dependent changes in CA3-CA1 network activity. Spike trains from selected CA3 and CA1 pyramidal neurons were then reiterated in dorsal hippocampal slices prepared from age-, sex- and strain-matched rats using whole-cell patch clamp recordings from CA1 pyramidal cells and synaptic responses elicited by stimulation of Schaffer Collateral (SC) axons. In addition to action potential firing, SC stimulation was used to simulate bursts of subthreshold synaptic input received by CA1 pyramidal cells during ripples (Maier, Tejero-Cantero et al. 2011). Replay activity patterns from CA3-CA1 place cell pairs coactivated during exploration of a novel environment are capable of inducing long-term potentiation. However, LTP is only expressed if replay patterns are delivered concurrently with simulated sub-threshold ripple oscillations.

This dependence of LTP on ripple-associated synaptic input suggests that the combination of sequential action potential firing coupled with ripple oscillations during quiescent behavioural states plays an enabling role in optimising the induction of synaptic plasticity likely to underlie memory consolidation.

References

Maier, N., A. Tejero-Cantero, et al. (2011). "Coherent phasic excitation during hippocampal ripples." Neuron 72(1): 137-152.

Disclosures: J. Sadowski: None. **J.R. Mellor:** None. **M.W. Jones:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.05/F14

Topic: B.08. Synaptic Plasticity

Title: Neuromodulators-mediated consolidation of STDP eligibility traces

Authors: *K. HE, A. KIRKWOOD;
Mind/Brain Inst., The Johns Hopkins Univ., Baltimore, MD

Abstract: Neuromodulators are thought to serve as reward signals in reinforcement-based and reward-based learning, including perceptual learning. A significant challenge for understanding this type of learning is the "credit assignment" or "distal reward problem": how synapses that were active before the arrival of the reward are modified. Theoretical studies postulate that the synaptic activity underlying learning 'tags' the synapses with a short-lived 'eligibility trace', by which the delayed reward identify the target synapses. Here we present experimental evidence to support this hypothesis. We found in the primary visual cortex (V1) that the spike-timing stimulation (correlated firing of pre- and postsynaptic neurons) can generate synapse-specific 'eligibility traces'. Stimulation of serotonergic and adrenergic receptors shortly afterwards consolidates these traces into either LTP or LTD depending on the STDP patterns as well as the modulators. We propose this neuromodulator-mediated consolidation of traces induced by STDP as a candidate cellular mechanism for reward-based perceptual learning.

Disclosures: K. He: None. **A. Kirkwood:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.06/F15

Topic: B.08. Synaptic Plasticity

Support: Wellcome Trust

Title: Spatiotemporal input dependent plasticity in cortical pyramidal cell dendrites

Authors: M. MACAK[1], T. BRANCO[2], *M. HAUSSER[1];
[1]UCL, London, United Kingdom; [2]MRC Lab. for Mol. Biol., Cambridge, United Kingdom

Abstract: Dendrites of pyramidal cells are known to be sensitive to distinct spatiotemporal patterns of inputs, which could be important for computations performed in sensory cortices. This sensitivity depends on the gradient of input impedance along dendritic branches and on NMDA receptor activation (Branco, Clark, & Häusser, Science 2010), whereby different temporal sequences recruit different amounts of NMDAR conductance. Given the well-known dependence of multiple forms of plasticity on NMDA receptors it is possible that different temporal sequences of synaptic inputs could result in differential plasticity. Such mechanism could, in theory, increase the computational power of dendrites and provide a biophysical basis for long-term storage of specific spatiotemporal input patterns. Here we investigated the possibility of sequence-dependent plasticity by combining 2-photon MNI-glutamate uncaging and whole-cell electrophysiological recordings from layer 5 pyramidal cells in acute slices of rat somatosensory cortex. Using high-frequency spike-timing dependent plasticity protocols (STDP), we first demonstrate that sequential synaptic activation along single dendritic branches can reliably result in timing-dependent long-term potentiation, in both apical (mean LTP = 208%, N=5) and basal dendrites (mean LTP = 185%, N=4). We are currently exploring the sensitivity of these plasticity mechanisms to different temporal sequences during STDP induction.

Disclosures: M. Macak: None. **M. Hausser:** None. **T. Branco:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.07/F16

Topic: B.08. Synaptic Plasticity

Support: FP7-ICT (Realnet)

Title: Reoccurring spatiotemporal optogentic stimulation pattern induces plasticity in a cortical neuron

Authors: *V. LERNER[1], S. ZIBMAN[1], H. SOMPOLINSKY[1,2], M. LONDON[1], Y. YAROM[1];
[1]The Edmond & Lily Safra Ctr. for Brain Sci., [2]Racah Inst. of Physics, Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: How does the brain identify and produce patterns of information required to process sensory information and to create motor commands? One possibility proposed by theoretical studies is that individual neurons can learn to respond to specific reoccurring spatiotemporal patterns of synaptic inputs that repeat in the presence of background activity. Several models have been suggested to explain both unsupervised (Masquelier et. al. 2008) and supervised (Gutig & Sompolinsky 2006) learning. All these models rely on synaptic plasticity to strengthen synapses associated with the desired input and weakening the others but there is yet no experimental evidence to determine the validity of the theoretical works.
We combine whole cell recordings in vitro with optogenetics and multispot illumination to address this question. We use Cop4 Thy-1 transgenic mice expressing Channelrhodopsin 2 (ChR2) sparsely in layer V somatosensory cortex. We record intracellularly from a cell not expressing ChR2. We create a grid of 100 illumination spots surrounding the neuron and map their individual postsynaptic response to light activation. We then generate spatiotemporal light patterns consisting of multi-site activation (1-10 spots) separated by 20-30 ms. The recorded cell is "trained" by a repetitive presentation of a specific spatiotemporal pattern embedded within a random sequence, as suggested by (Masquelier et. al. 2008). This type of massive activation results in profound plastic changes in the individual synaptic responses, which might lead to pattern recognition. This research is supprted by the FP7-ICT (Realnet) grant.
Gütig, R., & Sompolinsky, H. (2006). The tempotron: a neuron that learns spike timing-based decisions. Nature neuroscience, 9(3), 420-428.
Masquelier T, Guyonneau R, Thorpe SJ (2008). Spike Timing Dependent Plasticity Finds the Start of Repeating Patterns in Continuous Spike Trains. PLoS ONE, 3(1), e1377.

Disclosures: V. Lerner: None. **S. Zibman:** None. **H. Sompolinsky:** None. **M. London:** None. **Y. Yarom:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.08/F17

Topic: B.08. Synaptic Plasticity

Title: Natural firing patterns reduce sensitivity of synaptic plasticity to spike-timing

Authors: *S. OSTOJIC[1], M. GRAUPNER[2];
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Abstract: Synaptic plasticity is sensitive to both the rate and the timing of pre- and postsynaptic spikes. In experimental protocols used to induce plasticity, the imposed spike trains are regular and the relative timing between every pre- and postsynaptic spike is fixed. This is at odds with natural firing patterns observed in the cortex of intact animals, where cells fire irregularly and the timing between pre- and post-synaptic spikes varies.
To investigate synaptic changes elicited by in vivo-like irregularly firing neurons at different rates and realistic correlations between pre- and post-synaptic spikes, we use numerical simulations and mathematical analysis of synaptic plasticity models. We concentrate on a spike-timing model based on spike-pairs (Gerstner et al. 1996), spike-timing model based on spike-triplets (Pfister and Gerstner 2006), and further consider a calcium-based model (Graupner and Brunel 2012). The voltage-based model (Clopath et al. 2010) and the spike-triplet model (Pfister and Gerstner 2006) behave equivalently and we consider only the latter. To allow for comparison, all models are fitted to plasticity results obtained in vitro (Sjostrom et al. 2001).
We show that stimulation protocols with regular spikes and fixed relative timings overestimate the influence of spike-timing on synaptic plasticity. Using a simple modification of regular spike-pair protocols, we allow for neurons to fire irregularly. Such irregular spike-pairs reduce the amplitude of potentiation and depression obtained by varying the time difference between pre- and postsynaptic spikes. This protocol allows us to quantify the relative effects of firing rate and timing in natural firing patterns, and to predict changes induced by an arbitrary correlation function between pre- and post-synaptic spikes. We show that spike correlations considerably change synaptic plasticity at low firing rates in all models; as firing rates increase the influence of correlations remains strong in the spike-pair model, but diminishes in the triplet model and becomes negligible in the calcium-base model. Our findings yield predictions for novel experiments and help bridge the gap between existing results on synaptic plasticity and plasticity occurring under natural conditions.

Disclosures: S. Ostojic: None. **M. Graupner:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.09/F18

Topic: B.08. Synaptic Plasticity

Support: NIH grant EY-12782

Title: Role of experience in plasticity outcomes of spatially separate synaptic pathways onto individual neurons in mouse visual cortex

Authors: *O. M. FITCH[1,2], M. J. FRIEDLANDER[1];
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Abstract: In the neocortex, individual cells of like type can undergo heterogeneous plasticity responses from depression (LTD) to potentiation (LTP), or no change (NC) in response to a common fixed time delay synaptic conditioning protocol. However it is not known whether all synapses onto a common cell have the same plasticity outcome. Nor is it known what role visual experience plays in shaping the distribution of differential plasticity outcomes. Thus, we evaluated the synaptic plasticity responses of separable sets of synaptic inputs onto common postsynaptic neurons in primary visual cortex in response to simultaneous stimulation of distinct sets of afferents in acute brain slices from visually intact and in binocularly deprived mice. Animals were binocularly deprived from before the natural time of eye-opening. The two stimulation sites were isolated by occlusion testing followed by alternative activation of each pathway to evoke a postsynaptic potential (PSP) every 10 seconds in an interleaved fashion. After a stable ten minute baseline period, the activation of both pathways was simultaneously paired with direct postsynaptic activation that preceded the synaptic stimulation by 10 milliseconds resulting in 4-7 postsynaptic spikes at 0.1 Hz over a 10 min period followed by reversion to the interleaved stimulation protocol for an additional 30 minutes. The ratio of the average amplitude of the evoked PSP post/pre conditioning was calculated for each pathway taking the 5 minutes average peak amplitude over 25-30 minutes post-conditioning compared to 5 minutes just before or pre-conditioning. Our results from 57 pathways inputs validate in the mouse cortex our previous findings from other species demonstrating heterogeneous plasticity outcomes ranging from LTD to LTP for individual cells with a mean post/pre ratio of 0.87 +/- 0.32sd for the grouped results. Preliminary data comparing 30 pathways in the binocularly deprived animals also resulted in a similar range of individual plasticity outcomes with an average post/pre ratio of 0.88+/-0.40sd for the grouped results that was not significantly different from visually intact animals (p=0.95 t-test). When the plasticity outcomes of the separate pathways were compared to determine whether each set of synapses onto a common cell had similar plasticity behavior, we found that they were independent both in the visually intact animals with a $\chi 2$ of 1.6, 5 df, p=0.9 and in binocularly deprived animals $\chi 2$ of 5.6, 5 df, p=0.35. Thus, synaptic plasticity outcome variability is a local synaptic phenomenon vs. a whole cell property and appears to be intrinsic vs. modifiable by visual experience.
Supported by NIH grant EY-12782 to MJF.

Disclosures: O.M. Fitch: None. **M.J. Friedlander:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.10/F19

Topic: B.08. Synaptic Plasticity

Support: CIHR MOP-102617

CIHR New Investigator MOP-109357

Title: Twenty four hour sweetened high fat food treatment increases excitatory synapses onto vta dopamine neuron in mice

Authors: *S. LIU, S. BORGLAND;
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Abstract: Over-consumption of palatable food can lead to obesity. Dopamine neurons of the ventral tegmental area (VTA) are part of a critical circuit for reward seeking and hedonic feeding. Previously, we demonstrated that insulin can induce long-term depression (LTD) of VTA dopamine neurons via endocannabinoid-mediated presynaptic mechanism and this LTD can be occluded by a sweetened high-fat meal (SHF). In the present study, mice had 24 hour access to either SHF or regular food (RF). While caloric consumption was similar between RF and SHF mice, plasma insulin was significantly increased in mice fed SHF. Significant differences in the efficacy of excitatory synaptic transmission onto VTA dopamine neurons were observed between RF and SHF fed mice. Similar to 1h access to SHF, 24 hours of SHF increased endocannabinoid tone at excitatory synapses onto dopamine neurons. However, the co-efficiency variance of mEPSC amplitudes was significantly less in these mice, suggesting possible increased excitatory synapse formation onto dopamine neurons. These data suggest that synaptic depression induced by increased endocannabinoid tone may be offset by increased synapse formation. Consistent with this, there was no significant difference in mEPSC frequency and the latency to maximal spike-timing dependent long term potentiation (STDP) was significantly less in mice fed SHF. Taken together, these data suggest that 24 h access SHF results in synaptic plasticity at excitatory synapses in the VTA.

Disclosures: S. Liu: None. **S. Borgland:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.11/F20

Topic: B.08. Synaptic Plasticity

Support: KAKENHI 23240065 & 24650249

Title: Cultured cortical neurons can detect a higher-probability hidden signal source with multi-electrode stimulation

Authors: *T. ISOMURA, K. KOTANI, Y. JIMBO;
Dept. of Human and Engineered Envir. Studies, Grad. Sch. of Frontier Sci., The Univ. of Tokyo, Chiba, Japan

Abstract: Computational studies demonstrate that simulated neural networks can perform Principal component analysis (PCA); however, few studies have examined the neural basis of PCA-like learning. Here, we demonstrate that cultured cortical neurons can detect a higher-probability hidden signal source with multiple input signals. Approximately 500,000 dissociated cells obtained from the 19-day embryonic rat cortex were seeded on microelectrode array (MEA) dishes and cultivated for more than 3 weeks. We electrically stimulated the neurons with 256-s pulse trains that were applied at 1-s intervals. The stimulation was applied through 32 electrodes and was repeated for 100 cycles. These trains were constructed using two independent binary signal sources, u1(t) and u2(t). The values for half of the stimulus trains were randomly selected at each time period by u1(t) with a probability of 3/4, or by u2(t) with a probability of 1/4. The values for the remaining stimulus trains were randomly selected by u1(t) with a probability of 1/4, or u2(t) with a probability of 3/4. Neural responses evoked with the input trains were recorded with the 64-electrode MEA. Finally, we calculated the probability of the number of evoked spikes P(r). We observed a change in Kullback-Leibler divergence (KLD) between P(r|(u1,u2)=(1,0)) and P(r|(u1,u2)=(0,1)) as well as mutual information (MI) between P(r) and P(u1), or between P(r) and P(u2). For some electrodes, KLD increased (Fig.1) and one of the MIs with two hidden signal sources decreased after the training period, whereas KLD and MIs did not change significantly in the presence of 20 μM 2-Amino-5-phosphonopentanoic acid (APV) or without the stimulation. These results suggest that continuous stimulation induced synaptic plasticity in cultured neuronal networks, and that neurons located near some electrodes preferred to respond to a side of the hidden signal sources rather than respond to the input trains. Therefore, these results support the hypothesis that cortical neurons can detect the highest-probability or largest hidden signal source of input signals.

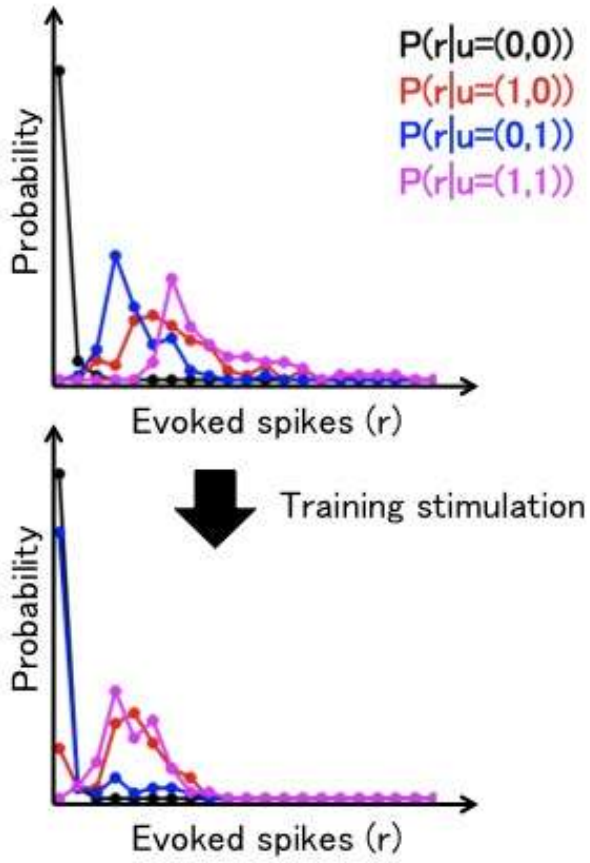

Fig.1. The probability of evoked spikes

Disclosures: T. Isomura: None. **K. Kotani:** None. **Y. Jimbo:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.12/F21

Topic: B.08. Synaptic Plasticity

Support: EU project FP7-269921 (BrainScaleS)

EU project FP7-248311 (AMARSi)

Title: Learning probabilistic inference in general graphical models with networks of spiking neurons

Authors: *D. PECEVSKI, W. MAASS;
Graz Univ. of Technol., Graz, Austria

Abstract: Many behavioral data as well as recent data in neuroscience [Berkes et al. 2011] suggest that the brain stores knowledge in form of probability distributions, which are then used to make inferences about the world based on observed facts. But it is still largely unknown how plasticity processes on a synaptic and neuronal level in the brain enable learning of knowledge in form of probability distributions. Recent theoretical results [Buesing et al., 2011, Pecevski et al., 2011] showed a novel way how a network of stochastic spiking neurons can "embody" a probability distribution as a distribution of network states, and perform probabilistic inference for it via Markov chain Monte Carlo sampling. Building on these results, we show here that a STDP-like synaptic plasticity mechanism together with plasticity of the intrinsic neuron excitabilities derived from basic theoretical principles, enable learning of a probability distribution in such networks of spiking neurons from input data streams. The approach assumes that there is prior knowledge about the existing independencies between the random variables in the probability distribution in the form of a graphical model structure. The neural networks are composed of interconnected winner-take-all network motifs with a connectivity that reflects the independencies in the graphical model and exploits these independencies to reduce the complexity of learning. The developed neural models are general in a sense that they can be applied for learning any probability distribution over discrete variables represented by any graphical model structure. We demonstrate the viability of the approach through computer simulations, where we train networks of spiking neurons to learn probabilistic models for two perceptual phenomena: perceptual explaining away and localization bias in multisensory integration. Altogether the results elucidate a novel integrated view of the functional role of synaptic plasticity, intrinsic plasticity and winner-take-all network motifs of spiking neurons: to support accumulation of knowledge in the probability distribution of network states.
References:
Berkes P, Orbán G, Lengyel M, and Fiser J (2011) Spontaneous Cortical Activity Reveals Hallmarks of an Optimal Internal Model of the Environment, Science, 331(6013), 83-87.
Pecevski D, Buesing L, Maass W (2011) Probabilistic Inference in General Graphical Models through Sampling in Stochastic Networks of Spiking Neurons. PLoS Comput Biol 7(12): e1002294.
Buesing L, Bill J, Nessler B, Maass W (2011) Neural Dynamics as Sampling: A Model for Stochastic Computation in Recurrent Networks of Spiking Neurons. PLoS Comput Biol 7(11): e1002211.

Disclosures: D. Pecevski: None. **W. Maass:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.13/F22

Topic: B.08. Synaptic Plasticity

Support: Stanley Center for Psychiatric Research at the Broad Institute

Picower Foundation

Singleton Fellowship (H.H)

Title: Neurogranin modulates the threshold for inducing LTP in spike-timing-dependent plasticity

Authors: *H. HWANG, K. J. JONES, W. XU;
Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Neurogranin (Ng) is a small neuron-specific protein, and primarily expressed in the postsynaptic compartments of projection neurons in hippocampus. Ng has been shown to interact with calmodulin (CaM) in vivo under low calcium conditions, and its binding to CaM reduces the calcium binding affinity of CaM. We found that Ng levels in hippocampus can be rapidly upregulated in response to novel context exposure and adrenergic stimulation. This upregulation can also be recapitulated in the neuronal culture system by elevated excitatory drive. Considering the role of Ng in binding CaM, such activity-dependent regulation of Ng levels may alter the dynamics of Ca2+/CaM complex formation and rises in the free calcium levels upon the entry of calcium ions. Thus, changes in Ng levels can critically influence Ca2+- or Ca2+/CaM-dependent neuronal processes such as synaptic plasticity.
Here we examined the role of Ng in inducing long-term potentiation (LTP) with a spike-timing-dependent plasticity (STDP) protocol in acute hippocampal slices. Ng levels were manipulated in mouse hippocampal CA1 neurons in vivo by stereotaxically injecting lentivirus that either overexpresses Ng fused to eGFP, or knocks down endogenous Ng with shRNA specifically targeting Ng mRNAs. STDP was induced by pairings of the Schaeffer collaterals stimulation with postsynaptic neuron bursting at various positive time intervals, based on a previously reported protocol (PNAS, 108, 2011, 8450-8455). Knockdown of endogenous Ng abolished spike-timing-dependent LTP (t-LTP) at a time interval that uninfected control neurons exhibited reliable, robust potentiation. Whereas overexpression of Ng significantly enhanced the magnitude of t-LTP at a time interval that uninfected neurons exhibited only mild potentiation. Taken together, our results demonstrate that Ng levels play a crucial role in setting the threshold/magnitude of LTP in STDP.

Disclosures: H. Hwang: None. **K.J. Jones:** None. **W. Xu:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.14/F23

Topic: B.08. Synaptic Plasticity

Support: NIH grant EY-12782

Title: The roles of individual Ca^{2+} sources in plasticity under different synaptic conditioning patterns in visual cortex

Authors: *D. KALIKULOV, M. J. FRIEDLANDER;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: We compared the contributions of NMDARs, RyRs, IP_3Rs, and mGluRs to induction of synaptic plasticity in visual cortex pyramidal neurons in response to two different conditioning paradigms. One used low frequency stimulation (LFS - 0.1 Hz) of afferents while the other employed pairing of presynaptic activity induced by stimulation of afferents 10 ms after postsynaptic spiking induced by intracellular current injection in young (P6-P12) guinea pigs. LFS resulted in a net LTD (-13±2%; n=10) while individual cells underwent LTD or no change (NC). When LFS was applied with inhibition of NMDA receptors (bath applied D-AP5 - DL-2-Amino-5-phosphonopentanoic acid) prevented induction of LTD (50 µM d-AP5,+0.3±3%, n=14), while LFS applied with inhibition of mGluRs or IP_3Rs with bath applied MPEP (6-Methyl-2-(phenylethynyl)pyridine - 10 µM, -14+5%, n=9) or xestospongin C (1.0µM, -17+6%, n=11) respectively, did not alter the likelihood or magnitude of LTD. However, when LFS was applied with inhibition of RyRs, (200 µM ryanodine) the plasticity outcome shifted to a net long term potentiation (LTP) (+27±14%, n=16). Application of the pairing protocol resulted in a range of plasticity outcomes (LTP, LTD or NC), with an overall net LTP (+9±5%, n=30) while individual cells underwent LTD, NC or LTP. When pairing was applied with selective inhibition of each of the individual calcium sources, there were significant changes in the plasticity outcomes ($p \leq 0.05$; t-test) compared to the plasticity outcomes in response to pairing under control conditions. A net LTD occurred in the presence of inhibitors of NMDARs (50 µM d-AP5, -36±6%, n=20), or in the presence of inhibition of RyRs (200µM ryanodine, -31±6%, n=20). Conversely, a net LTP occurred in the presence of inhibitors of mGluRs (10 µM MPEP, +31±8%,n=27) or IP_3Rs (1.0µM xestospongin C, +31±9%, n=25). Evaluation of the plasticity outcome distributions across individual cells showed that inhibition of NMDARs or RyRs, resulted in LTD or NC, while inhibition of mGluRs or IP_3Rs resulted in NC or LTP. These results suggest that NMDAR activation is necessary for LFS stimulation induced LTD while

mGluR and IP_3R activation are not, and equivalent pairing of pre- and postsynaptic activity can access relatively different contributions of the various postsynaptic calcium signaling pathways in different neurons of like type under otherwise identical conditions. These differences may be due to intrinsic variability in the expression and/or compartmentalization of these calcium sources and/or the state or past experience of the individual cells/synapses.
Supported by NIH grant EY-12782 to MJF

Disclosures: D. Kalikulov: None. **M.J. Friedlander:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.15/F24

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01-DC009215

Title: Characterizing topology-dependent network plasticity induced by electrical stimulation in silico

Authors: *R. NI, D. B. SINHA, N. M. LEDBETTER, D. L. BARBOUR;
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Abstract: Activity-dependent electrical stimulation can induce cortical reorganization in vivo by activating brain areas using stimulation derived from the statistics of neural or muscular activity. Regular failures of network-level reorganization, however, have been observed in experiments applying such stimulation protocols. These procedures were designed based upon the nature of monosynaptic Hebbian plasticity, but induced reorganization is likely to involve polysynaptic circuits. Unfortunately, the network manifestation of plasticity across multiple synapses is poorly understood. We hypothesize that the regular failures in stimulation-induced reorganization may be attributable to variations in network topology. In this study, we explored and characterized the influence of local neural circuit topology on the effectiveness of activity-dependent electrical stimulation in modulating synaptic strength. We simulated small-scale neural networks possessing feedforward and recurrent topologies, as well as the same small networks embedded within larger networks. Excitatory synapses were modulated by an unbounded but weight-dependent spiking-timing-dependent plasticity (STDP) rule. We used one type of activity-dependent electrical stimulation, spike-triggered stimulation (STS), to activate single neurons or groups of neurons based upon the ongoing activity of a reference neuron. The results demonstrate that STS-induced competition between the conditioned input and network synaptic

inputs yielded a new steady-state synaptic strength distribution that emerged during stimulation. Under some experimental conditions, STS elevated mean network firing rate and increased the mutual information between conditioned neurons and the unconditioned neurons. Stimulating a group of neurons rather than individual neurons revealed a wider range of information rerouting. STS effects for paired neurons with direct connections were generally large and could persist following the stimulation period in both small and larger neural networks. For paired neurons without direct connections, the effects of STS in small local circuits rapidly washed out after stimulation ended but were somewhat persistent in larger scale networks. This study provides insights into the understanding of the impact of local circuit topologies on activity-dependent stimulation, as well as polysynaptic plasticity, and will ultimately facilitate the effective manipulation of brain networks with activity-dependent stimulation.

Disclosures: R. Ni: None. **D.B. Sinha:** None. **N.M. Ledbetter:** None. **D.L. Barbour:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.16/F25

Topic: B.08. Synaptic Plasticity

Support: EuroSPIN Erasmus Mundus Doctoral Programme

FP-7 BrainScaleS 269921

MRC Fellowship G0900425

Swedish e-science Research Center

Swedish Research Council

Stockholm Brain Institute

Title: A modular attractor memory network with spike-based, probabilistic learning

Authors: P. J. TULLY[1,2,3], M. H. HENNIG[3], *A. B. LANSNER[4,1,2];
[1]Dept. of Computat. Biol., Royal Inst. of Technol. (KTH), Stockholm, Sweden; [2]Stockholm Brain Inst., Karolinska Institutet, Stockholm, Sweden; [3]Inst. for Adaptive and Neural Computation, Univ. of Edinburgh, Edinburgh, United Kingdom; [4]Numerical Analysis & Computer Sci., Stockholm, Sweden

Abstract: A rich set of computations emerges from the dynamics of networks of recurrently connected neurons. The attractor-memory paradigm provides a powerful yet simple approach to understanding how these dynamics could enable many aspects of cortical function [1], but this abstract mapping often occurs at the expense of biological plausibility. In these models, each memory is stored in a distributed fashion represented by increased firing in pools of excitatory neurons. Excitatory activity is locally modulated by inhibitory neurons representing lateral inhibition causing winner-take-all dynamics. Detailed network models of this type have previously been shown to exhibit switching between non-coding ground state and low-rate memory state activations displaying gamma oscillations [2], however it is unclear how such properties could be shaped by on-line Hebbian plasticity. Assuming a probabilistic framework in which local neuron populations discretely encode uncertainty about an attribute in the external world (e.g. a column in visual cortex tuned to a specific edge orientation), we seek to bridge this gap by modeling inter-module synapses using the Bayesian Confidence Propagation Neural Network (BCPNN) plasticity rule [3].
We use a spike-based version of BCPNN in which synaptic weights are statistically inferred by estimating the posterior likelihood of activation for the postsynaptic cell upon presentation of evidence in the form of presynaptic activity patterns. Probabilities are estimated on-line using local exponentially weighted moving averages, with time scales that are biologically motivated by the cascade of events involved in the induction and maintenance of long-term plasticity. Modulating the kinetics of these traces is shown to shape the width of the STDP kernel, which in turn allows attractors to be learned forwards or backwards through time. Stable learning is confirmed by a unimodal stationary weight distribution. Inference additionally requires modification of a distinct neuronal component, which we interpret as a correlate of intrinsic excitability. Incorporation of the learning rule allows for continuous real-time learning in the spiking attractor network.
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[2] Lundqvist, M., Compte, A., & Lansner, A. (2010) Bistable, irregular firing and population oscillations in a modular attractor memory network. PLoS Comput Biol 6(6).
[3] Lansner, A. & Ekeberg, Ö. (1989). A One-Layer feedback artificial neural network with a Bayesian learning rule. Int. J. Neural Syst 1, 77-87.

Disclosures: P.J. Tully: None. **M.H. Hennig:** None. **A.B. Lansner:** None.

Poster

519. **Spike-Timing Dependent Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 519.17/F26

Topic: B.08. Synaptic Plasticity

Title: Plasticity of excitatory-inhibitory balance in the auditory cortex

Authors: *J. A. D'AMOUR, R. C. FROEMKE;
Mol. Neurosci., New York University, Sch. of Med., New York, NY

Abstract: In mature neuronal networks, excitatory and inhibitory responses are correlated, such that inputs that evoke large excitatory responses tend to evoke large inhibitory responses, and small excitatory responses are usually accompanied by small inhibitory responses, i.e., excitation and inhibition are balanced. While it is known that excitatory synapses are modified in an activity dependent manner, it remains unclear how inhibitory responses are modified following excitatory plasticity to restore balance. We have previously examined this phenomenon in vivo in adult (Froemke et al., Nature 2007) and developing rat auditory cortex (Dorrn et al., Nature 2010). Here we look at the synaptic mechanisms of excitatory-inhibitory balancing in young and adult mouse auditory cortex in brain slices, in order to examine this process in higher resolution. We made whole-cell recordings from layer 5 neurons in slices of mouse auditory cortex (postnatal day 12-24). First, we recorded from pyramidal cells and evoked excitatory or inhibitory responses with focal extracellular stimulation using an array of 4-8 stimulation electrodes to activate several point sources of excitatory and inhibitory inputs. After monitoring baseline responses, extracellular stimulation of one input channel was repetitively paired with a single postsynaptic spike for several minutes to induce spike-timing-dependent plasticity (STDP), and synaptic strengths of the entire population of 4-8 channels monitored thereafter. For excitatory inputs, pre-before-post pairing at short timing intervals (< 15 msec) induced long-term potentiation (LTP), while post-before-pre pairing induced long-term depression (LTD), as expected. These modifications were blocked by the NMDA receptor antagonist APV. Inhibitory STDP, however, showed a different learning rule from that of excitatory synapses, where-in both very short positive and negative pairings potentiated inhibitory responses, as predicted from a recent theoretical study (Vogels et al., Science 2011).
Modifications were also observed to unpaired inputs. We measured the effect of these homosynaptic and heterosynaptic changes by measuring the linear correlation between excitation and inhibition across the different input channels. When correlation was initially moderate to high ($r>0.4$), synaptic modifications had little positive effect on the post-pairing correlation. However, in 3/3 cases, when correlation began low (~0.3 or less), spike pairing at one input led to changes that collectively improved the overall correlation. We are now assessing the cellular mechanisms by which heterosynaptic modifications are induced.

Disclosures: J.A. D'Amour: None. **R.C. Froemke:** None.**Poster**

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.01/F27

Topic: B.09. Network Interactions

Support: NRF grant 2011-0014481

NRF grant R31-10008

Title: Feedforward and feedback inhibition differentially control the spike output patterns of CA1 pyramidal neuron

Authors: H. JANG, *J. KWAG;
Dept. of Brain and Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: Hippocampal pyramidal neuronal spike output patterns contain spike rate and temporal codes that are important in hippocampal spatial information processing. However, how the interneuronal network supports the generation of the two distinctive types of neural codes is yet unclear. Here, we investigated how the feedforward (FF) and feedback (FB) inhibitory network differentially modulate the spike output patterns of CA1 pyramidal cell (PC) by studying the input-output relation in a hippocampal network model.
The hippocampal network model consisted of a conductance-based CA1 PC receiving an afferent excitatory input from a CA3 PC. FF inhibition to CA1 PC was activated by the CA3 PC whereas FB inhibition to CA1 PC was activated by the recurrent inhibition activated by the CA1 PC. Input-output relation was quantified by analyzing the output frequency of a CA1 PC in response to the activation of CA3 PC at 2-100 Hz in each of the excitatory, FF and FB inhibitory network. In excitatory network, increasing the input frequency linearly increased the output frequency of CA1 PC (regression slope, a= 0.288). In the FB network, input-out relation also linearly increased, but the relation down-shifted (a = 0.200). In FF network, however, regardless of the input frequency, the output frequency was kept almost constant in the theta-frequency range (4-10 Hz, a = 0.0350). Temporal dynamics of the spike output was quantified by analyzing the inter-spike interval (ISI) and its coefficient of variation (CV). ISI of excitatory network and FB network showed small variation (CV=0.319 and 0.2284, respectively). Especially, FB inhibitory network showed a near constant ISI, suggesting that FB inhibitory network promotes synchronization of spike output patterns. However, FF inhibitory network showed comparably variable ISI with the greatest CV among the three network conditions (CV = 0.407).
Here we demonstrate that FF and FB inhibitory network differentially control spike output patterns of CA1 PC. FB inhibitory network may support synchronization of spike outputs promoting rate coding whereas FF inhibitory network may contribute to temporal coding in the theta-frequency range, suggesting that different inhibitory connections may have different functions in hippocampal information processing.

Disclosures: H. Jang: None. **J. Kwag:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.02/F28

Topic: B.09. Network Interactions

Support: IWT Flander Research Fellowship 00000300661

Title: Multichannel recordings in the medial prefrontal cortex during carbachol and group I metabotropic glutamate receptor activation

Authors: *M. POLLARD, H. SHABAN;
Janssen Pharmaceutica NV, Beerse, Belgium

Abstract: Neural plasticity deficits that involve group I metabotropic glutamate receptors (mGluRs) relate to cognitive decline in Fragile X Syndrome. Also, boosting of NMDA receptor-mediated currents by mGluR subtype 5 is relevant in the hypoglutamatergic hypothesis for schizophrenia. The advent of positive allosteric modulators (PAMs) that enhance receptor-mediated activation, while avoiding alterations in endognenous neurotransmission has led to studies that report mGluR5 PAMs as beneficial in rodents during cognitive tasks. We are interested in understanding the neuronal circuit dynamics responsible for group I mGluR-mediated effects in the medial prefrontal cortex, an area important in cognitive control. Moreover, we will determine the interplay with the arousal agent, carbachol (CCH) as an assessment for therapeutic potential during behavioral states. Multichannel extracellular recordings were performed in acute coronal slices of rat brain placed in a biochip (8x8 array; 40 μm electrodes; 200 μm spacing). Control consisted of Ringer's solution and drugs were perfused for 5 min prior to recordings for 1 min at 21°C. Spontaneous IPSCs were recorded separately in layer V principal cells at -70 mV with a high chloride internal solution. Action potential and IPSCs are presented as percent changes of the mean ± SE with respect to control levels and $p < 0.05$ with Student's t test. Results show that CCH (20 μM) or the group I mGluR agonist, DHPG (100 μM) caused an increase spread of spiking activity (11.45 ± 0.04%; 9.17 ± 0.01%; n = 80; $p < 0.05$, respectively). Either CCH or DHPG caused increases in IPSCs, which were not significant. The mGluR5 PAM, VU-29 (1 μM) caused no effect on its own though the mGluR5 antagonist, MTEP (10 μM) resulted in increased spiking activity (23.77 ± 0.02%; n = 20; $p < 0.05$) indicating that mGluR5-mediated basal transmission was present but constrained. Interestingly, no changes in IPSCs were observed following MTEP, however, VU-29 caused a

large enhancement (259.41 ± 104.52%; n = 17; $p < 0.05$) of CCH-evoked IPSCs. When combining CCH (14.11 ± 0.11%) with VU-29 or MTEP, spiking rate decreased (7.48 ± 0.11%) or increased respectively (84.10 ± 0.30%; n = 20; $p < 0.05$). In contrast, combinations with DHPG (55.15 ± 0.12%) resulted in enhanced spread of activity for both VU-29 (64.00 ± 0.12%) and MTEP (90.61 ± 0.15%; n = 30; $p < 0.05$) without affecting spike rate. These data implicate a role for mGluR5-mediated feedforward inhibition in maintaining signal to noise ratios for relevant inputs during attention. It also emphasizes the influence of neuronal circuits in dictating response outputs important for assessing the effects of drug candidates.

Disclosures: M. Pollard: A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV. **H. Shaban:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.03/F29

Topic: B.09. Network Interactions

Title: Neural circuit that computes direction selectivity in mouse retina

Authors: J. S. KIM[1], M. GREENE[1], A. ZLATESKI[1], M. RICHARDSON[1], M. BALKAM[1], M. PURCARO[1], *H. SEUNG[1], M. HELMSTAEDTER[2], K. BRIGGMAN[2], W. DENK[2]; [1]Brain & Cog Sci. Dept., MIT, CAMBRIDGE, MA; [2]Max Planck Inst. of Neurobio., Munich, Germany

Abstract: The J retinal ganglion cell (J-RGC) is a genetically identified ganglion cell type in mouse retina that shows direction selectivity. We studied subcellular and intercellular organization of the direction selective circuit involving the J-RGC. With help of EyeWire, a community of citizen neuroscientists, we reconstructed a few J-RGCs and their synaptic inputs from serial electron microscopic images. We found that type 3/4 bipolar cells and starburst amacrine cells synapse primarily onto the dendrites of J-RGC proximal to its soma, while inputs from type 1/2 bipolar cells are dominate on the distal J-RGC dendrites. Such wiring specificity is a consequence of subtle differences in the stratification of proximal and distal dendrites of J-RGCs. Based on the observation, we introduce an excitory/inhibitory circuit model for direction selectivity. The study suggests that intercellular wiring wiring specificity is governed by subcellualar structures of different types of cells and that the visual function of J-RGC is intimately related to the morphology of itself as well as its circuit structure.

Disclosures: J.S. Kim: None. **M. Greene:** None. **A. Zlateski:** None. **M. Richardson:** None. **M. Balkam:** None. **M. Purcaro:** None. **H. Seung:** None. **M. Helmstaedter:** None. **K. Briggman:** None. **W. Denk:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.04/F30

Topic: B.09. Network Interactions

Support: Swiss National Science Foundation Ambizione Grant PZ00P3_132245

FP7 of the European Community through the ERC Advanced Grant 267351 "NeuroCMOS"

Japanese Society for the Promotion of Science post-doctoral grant

KAKENHI Grant 23680050

RCAST internal grant

Denso Corp.

Title: Local differences in axonal action potential conduction velocity

Authors: *D. J. BAKKUM[1], U. FREY[2], M. RADIVOJEVIC[1], J. MUELLER[1], M. FISCELLA[1], H. TAKAHASHI[3], A. HIERLEMANN[1];
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Abstract: Accumulating evidence shows that neocortical axons may play more important roles in neural computation than generally considered, namely, by regulating action potential propagation. For example, previous work demonstrated that neurons could temporally tune the propagation of their action potentials between two sites (Bakkum et al. 2008): Activity-dependent plasticity occurred as networks adapted to patterns of electrical stimulation and was regulated on the minutes to hours time scale relevant for some learning and memory processes. In this abstract, studies of axonal function were continued by taking advantage of recent developments in micro-electrode array (MEA) technology, which has provided the ability to track propagating action potentials traveling across hundreds of sites by using stimulus-triggered averaging. Rat cortical neurons and glia were cultured over a novel MEA containing 11,011

densely-packed electrodes within 3.4 square mm (Frey et al. 2010). Live-cell images of single axons transfected with fluorescent protein were compared to the electrophysiological signals they produced. Many-fold velocity differences existed between neighboring segments (<10s of microns), and the velocity profile temporally varied across days. Future experiments will address what mechanisms could be regulating action potential conduction locally and whether or not this is important for neural information processing.

Disclosures: D.J. Bakkum: None. **U. Frey:** None. **M. Radivojevic:** None. **J. Mueller:** None. **M. Fiscella:** None. **H. Takahashi:** None. **A. Hierlemann:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.05/F31

Topic: B.09. Network Interactions

Support: NIH Grant NS063494

Title: Propagation of errors in metabolically constrained networks of excitatory and inhibitory neurons

Authors: *J. CHAPETON, R. GALA, A. STEPANYANTS;
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Abstract: Long-term memories are stored in the strengths and patterns of synaptic connections. A memory of a sequence of events that plays out in time is recalled as the activity of neurons progresses from one network state to the next. In the absence of noise, such a memory sequence can be retrieved in its entirety. However, the stochastic nature of synaptic transmission can cause neurons to fire spontaneously or fail to generate action potentials. Clearly, memory retrieval has to be robust with respect to such errors; yet, it is not known how this robustness is achieved in cortical circuits of excitatory and inhibitory neurons. Thus, we examine the following questions. (1) Are the firing statistics of biological neural networks consistent with a regime in which errors are suppressed and do not disrupt memory retrieval? (2) How much noise can be tolerated by local cortical circuits during the memory retrieval process?
In order to quantitatively explore these questions we consider the model of steady-state learning. In this model a given cortical circuit in the adult functions in the state of maximal memory storage capacity, and learning of new memories is accompanied by forgetting of some of the old ones. Specifically, we analyze the retrieval of temporal sequences of associative memories stored in a network of robust McCulloch and Pitts neurons. During memory storage the neurons learn

by modifying the weights of their excitatory and inhibitory inputs in the presence of two biologically inspired constraints: (i) the firing thresholds of all neurons are kept constant, and (ii) the overall weight of connections received by each neuron (the L_1 norm) is fixed. As a result of the analysis we delineate the domain of excitatory and inhibitory firing frequencies in which memories can be recalled robustly.

Disclosures: J. Chapeton: None. **R. Gala:** None. **A. Stepanyants:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.06/F32

Topic: B.09. Network Interactions

Support: OTKA K81354

ANR-TÉT Neurogen

ANR-TÉT Multisca

TÁMOP-4.2.1.B-11/2/KMR-2011-0002

TÁMOP-4.2.2./B-10/1-2010-0014

FP7 Neuroseeker

Title: Two distinct origin of the active phases of the slow cortical rhythm in the somatosensory cortex of the ketamine-xylasine anesthetized rat

Authors: *R. FIÁTH[1], P. BEREGSZÁSZI[2], I. ULBERT[1,2];
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Abstract: The slow cortical rhythm (SCR) emerges during slow-wave sleep or under anesthesia. The cellular counterpart of the SCR, the slow oscillation is characterized by rhythmic alternation of two phases: the active phase (up-state) with strong synaptic activity, massive cell firing and depolarized membrane potential, and the inactive phase (down-state) with hyperpolarized cells and neuronal silence. Current knowledge suggests that the SCR is mostly of cortical origin, spontaneous up-states may initiate at any point of the neocortex and the rhythm behaves as a traveling wave. Recent results obtained with optogenetic methods indicate a more prominent role

of the infragranular cortical layers in the generation of the SCR compared to supragranular layers: synchronous activity of a small cluster of mouse layer V pyramidal cells is able to start a new active phase, while layer III pyramidal cells do not have this ability. Based on the observations of other research groups, up-states can be evoked with brief sensory stimuli through the thalamorecipient layer IV as well. However, we assume that up-states initiate also spontaneously from layer IV, in the absence of stimulation. Our assumptions are supported by the fact that the cortex and thalamus form a complex, reciprocally connected network and by previous studies that state that the thalamus may have an important role in the generation of the SCR.

To test our hypothesis, we recorded the local field potential and the multiple-unit activity from the somatosensory cortex of ketamine-xylazine anesthetized rats with a laminar silicon multielectrode. Cortical layers were identified on the Nissl-staind coronal slices and assigned to the appropriate recording channels. For every up-state the layer with the earliest unit activity was computed.

Our preliminary results show that in a notable part of the experiments, beside up-states starting in layer V, are also a significant amount of active phases initiated in layer IV present. Furthermore, in some cases the number of spontaneously occuring up-states in layer IV exceeded the number of up-states with layer V onsets.

Based on these results we can conclude that in certain regions of the rat somatosensory cortex active phases of the SCR can originate spontaneously not only from layer V, but also from layer IV. These latter up-states are generated presumably in the thalamus and relayed to the cortex. However, it is possible that one part of these spontaneous, layer IV up-states are initiated by sensory stimuli coming from the outside world which are not registered during the experiments, e.g. the continuous mechanical stimulation of the skin on the trunk of the animal caused by breathing.

Disclosures: R. Fiáth: None. **I. Ulbert:** None. **P. Beregszászi:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.07/F33

Topic: B.09. Network Interactions

Support: NIH/NIGMS MBRS-RISE GM060655

NSF EF-1137897

Title: The effects of propagation delays on spiking activity

Authors: *K. Y. TERRAZAS[1], W. TEKA[2], F. SANTAMARIA[3];
[1]Univ. of Texas At San Antonio, San Antonio, TX; [3]Biol., [2]Univ. of Texas at San Antonio, San Antonio, TX

Abstract: We are investigating the effects of axonal propagation velocities on coordinated transmission of synaptic information. For this purpose we have coded a computer program to simulate leaky integrate and fire neurons of different network sizes, connectivity, and distribution of axonal delays. Our modeling environment allows the implementation of arbitrary connectivity and propagation delays. The delay matrix is implemented as a three-dimensional buffer where instantaneous activity of the network is translated along the time axis. Such an environment allows us to investigate not only the traditional parameters of weight and network topology but also different distributions of delay times. The simulation environment is programmed in Matlab and has been compiled to run in parallel. We are particularly interested in studying how power-law axonal delays affect propagation of information in neural networks. First we have connected a neuron to an increasing number of cells that feedback to the main neuron with different types of delay distributions. We have calculated the firing rate properties and the cross and auto-correlations. Our results show the cumulative effects of long range correlations imposed by the network connectivity.

Disclosures: K.Y. Terrazas: None. **W. Teka:** None. **F. Santamaria:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.08/F34

Topic: B.09. Network Interactions

Support: BOF NOI Grant 2009

Title: Ultra-fast neuronal ensemble reaction times in cortical neurons under recreated synaptic background activity

Authors: *I. BIRO[1], D. LINARO[1], M. GIUGLIANO[1,2,3];
[1]Biomed. Sci., Univ. of Antwerp, Wilrijk, Belgium; [2]Brain Mind Inst., Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [3]Dept. of Computer Sci., Univ. of Sheffield, Sheffield, United Kingdom

Abstract: It has been shown that cortical ensembles, comprised of few thousand cells, can unexpectedly track fast varying input signals up to around 200 cycles/sec. They do so by relaying fast inputs downstream, in their spike trains. Such an input-output transfer bandwidth is in fact much wider than the limiting cutoff frequency imposed by the passive properties of individual neurons membrane (50 cycles/sec). This was first experimentally demonstrated by our group in 2008, performing in vitro a "linear system analysis" of output firing rates in response to current injection of sinusoidally modulated waveforms. These rapidly changing inputs were superimposed to randomly fluctuating signals, mimicking spontaneous synaptic activity observed in vivo.
However, the physiological activation of synaptic inputs to a neuron is mediated by ionotropic synaptic receptors (e.g., AMPAr, NMDAr, GABAa). This results not only in excitatory and inhibitory net currents, but also in a stimulus-dependent change of the overall effective membrane conductance of the postsynaptic cell. In order to include and study these effects in our experiments, we employed a real-time computer-controlled protocol (i.e., dynamic-clamp) to study input-output response properties of rat somatosensory cortical neurons from the layer 5 in acute brain slices. We asked whether neurons are able to track current- or conductance-based signals on a noisy conductance background. Our results clearly indicate that neurons receiving a barrage of emulated synaptic inputs, closely resembling the in vivo high-conductance state, have ultra-fast reaction times and track fast changing inputs. The input-output transfer bandwidth shows a similar upper limit of 200 cycles/sec, in both cases of current- and conductance-based signals. However, the effect of the background synaptic activation becomes prominent for slow varying input components, while it only marginally affects the firing rate modulation by fast varying components. Beyond the upper limit of the transfer bandwidth, background synaptic activity has no significant effect, as opposed to what reported in previous theoretical studies. The analysis of the input-output response phase-shift is also similar, comparing current- or conductance-based signals. In conclusion, we confirmed the validity of previous studies and extend them to more realistic physiological conditions.

Disclosures: I. Biro: None. **D. Linaro:** None. **M. Giugliano:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.09/F35

Topic: B.09. Network Interactions

Support: NIH Grant T32 DC000011

NIH Grant R01 DC000288

Title: Optogenetic investigation of local inhibitory circuitries in the nucleus of the solitary tract

Authors: *J. A. CORSON[1], R. M. BRADLEY[2];
[2]Biologic and Materials Sci., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: The rostral nucleus of the solitary tract (rNTS) is the first central target of primary orosensory nerve fibers. As such, it plays an essential role in the processing and coding of taste, tactile, and temperature sensory information from the oral cavity. The intrinsic circuitry within rNTS is likely integral in shaping the incoming information into both ascending (pontine-thalamo-cortical) and descending (intramedullary) efferent signals. Substantial subpopulations of interneurons in the rNTS are GABAergic and thus contribute to the transformation of incoming sensory afferent barrages into temporally modified spike trains. Despite this importance in shaping rNTS orosensory-evoked signaling, the organization of rNTS GABAergic circuits is unknown. To investigate the spatial patterning of GABAergic neurons presynaptic to identified subpopulations of neurons, we used a mouse model in which channelrhodopsin was expressed under the control of vesicular GABA transporter. Animals received small pressure injections of fluorescent microspheres into either the parabrachial nucleus or subjacent reticular formation to label either ascending or descending neurons respectively. Acute slices were prepared from these animals for in vitro patch clamp recording. GABAergic interneurons were activated with 473 nm laser illumination merged into the optical axis of the microscope. Focused laser illumination produced consistent saturated photocurrents in GABAergic neurons with high temporal and spatial resolution. While recording inhibitory postsynaptic currents in different rNTS neuron populations, the laser spot was systematically scanned over discrete portions of the rNTS to map out the GABAergic innervation onto the recorded neuron. Inhibitory maps will be compared for ascending and descending projection neurons as well as GABAergic interneurons. Neurons received inhibitory innervation from wide expanses of rNTS, often with focal spots of strong inhibition located in areas not immediately adjacent to the recorded neuron. This suggests that in addition to a low level of global inhibition, there are also specific subregions of rNTS that are able to strongly hyperpolarize individual neurons. This strong inhibition will likely induce alterations in repetitive discharge patterns through the numerous hyperpolarization-activated currents expressed in many rNTS projection neurons. The results of this study will provide valuable information into the mechanisms by which the rNTS is able to transform incoming sensory information into discrete efferent signals.

Disclosures: J.A. Corson: None. **R.M. Bradley:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.10/F36

Topic: B.09. Network Interactions

Support: JST-ERATO

Title: Fast voltage sensitive dye imaging of functional connectivity in the rat retrosplenial cortex

Authors: *K. NIXIMA[1,2,3], T. KUROTANI[2,3], K. OKANOYA[1,2,3];
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Abstract: The rodent granular retrosplenial cortex (GRS) has dense reciprocal connections with the anterior thalamic nuclei (ATN) and the hippocampal formation, hence playing a crucial role in some forms of learning and memory. In superficial layers of the GRS, distinctive pyramidal neurons, showing a late-spiking (LS) firing property, form prominent dendritic bundles in layer 1a co-localizing with the ATN termination patches. However, most previous interests were addressed to the anatomical aspects, and the detailed information about the functional connectivity within the GRS remains to be elucidated.
In this study, we conducted fast voltage sensitive dye (Di-4-ANEPPPS) imaging from slice preparations of the rat GRS, in order to clarify the detailed intracortical signal transmissions. In the coronal plane, layer 1a stimulation evoked excitatory synaptic transmission in layers 2-4 and subsequent excitation in layers 5-6. Each excitation in those layers was followed by transverse signal propagation within the layers, and spread to the dysgranular retrosplenial cortex (DRS). Interestingly, either superficial or deep layer stimulation induced monosynaptic inhibitory responses mainly in layers 2-4, which was confirmed by perfusing glutamate antagonists, DNQX and DL-APV. In the horizontal plane, layer 1a stimulation evoked similar spatio-temporal patterns. Compared with the coronal slices, layer 1a stimulation induced broader transverse signal propagation in deep layers. This propagation was not disrupted by a perpendicular cut in the deeper layers, indicating that the excitation was transferred by way of the superficial layers. The direction of the excitation propagation was predominantly from posterior to anterior, usually with less inhibitory responses compared with those observed in the coronal slices. In contrast to the layer 1a stimulation, the subiculum or corpus callosum stimulation mainly activated deep layer neurons of the GRS rather than superficial neurons.
In conclusion, signal inputs from the ATN can be conveyed to layers 5-6 neurons (and then to the neocortex or frontal robes), by LS-neuron network in superficial layers. Since the LS-neuron network is suggested to have a filtering role for the ATN signals, layers 5-6 neurons are likely to integrate filtered signals from the ATN with non-filtered (direct input) signals from the hippocampal formation. The spatio-temporal dynamics of neural activities in the GRS microcircuitry may thus be important for learning and memory.

Disclosures: K. Nixima: None. **T. Kurotani:** None. **K. Okanoya:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.11/F37

Topic: B.09. Network Interactions

Support: San Paolo "Programma in Neuroscienze"

MIUR FIRB (RBAP11X42L)

Telethon-Italy (GGP10138)

Title: Layer V photo-stimulation efficiently recruits infragranular and supragranular cortical neurons

Authors: R. BELTRAMO, P. FARISELLO, *T. FELLIN;
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Abstract: Recent *in vivo* reports demonstrate that layer V pyramidal neurons are fundamental in the regulation of the spontaneous network activity and that they efficiently propagate prolonged depolarizations within and across cortical layers. Since layer V pyramidal cells represent a major output of the cortical column and project to both cortical and extra-cortical areas, the spread of depolarization that follows the activation of layer V pyramids might be mediated by intra- or extra-cortical fibers. To discriminate between these two possibilities, we performed patch-clamp recordings in acute cortical slices in which the cortical circuitry was isolated from the rest of the brain. Using the transgenic mouse line *Rbp4-Cre,* which expresses the enzyme Cre in a subset of layer V neurons, we conditionally expressed the light-gated cation channel channelrhodopsin-2 (ChR2) through adeno-associated viral injections. We recorded in current-clamp configuration the responses of ChR2-negative neurons located in infragranular and supragranular layers, while stimulating the subpopulation of ChR2-positive cells in layer V. At their resting membrane potential, all recorded neurons responded to light stimulation with complex membrane depolarizations which were characterized by supra-millisecond latencies. These preliminary experiments suggest that intra-cortical projections are sufficient to propagate within and across cortical layers the depolarizing events that follow layer V photo-activation.

Disclosures: R. Beltramo: None. **P. Farisello:** None. **T. Fellin:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.12/F38

Topic: B.09. Network Interactions

Title: Entorhinal cortical theta-frequency input to the dentate gyrus trisynaptically evokes hippocampal CA1 LTP *In vitro*

Authors: *J. STEPAN[1], J. DINE[1], T. FENZL[6], S. A. POLTA[2], A. URIBE[3], G. VON WOLFF[4], F. HOLSBOER[5], M. V. SCHMIDT[3], C. T. WOTJAK[2], M. EDER[1];
[1]Neuronal Network Dynamics, [2]Neuronal Plasticity, [3]Neurobio. of Stress, [4]Neuroimaging, [5]Max Planck Inst. of Psychiatry, Munich, Germany; [6]Dept. of Pharmacol. and Toxicology, Leopold-Franzens-University, Innsbruck, Austria

Abstract: There is substantial evidence that some forms of explicit learning in mammals require long-term potentiation (LTP) at hippocampal CA3-CA1 synapses. While CA1 LTP has been well characterized at the monosynaptic level, it still remains unclear how the afferent systems to the hippocampus can initiate formation of this neuroplastic phenomenon. Using a combination of fast voltage-sensitive dye imaging (VSDI) and field potential recording in an elaborate mouse brain slice preparation, we show that stimulus-evoked entorhinal cortical (EC) theta-frequency (5 Hz) input to the dentate gyrus highly effectively generates waves of neuronal activity which propagate through the entire trisynaptic circuit of the hippocampus ("HTC-Waves"). This flow of activity is markedly less pronounced during 1 and 20 Hz EC input and not inducible using a stimulation frequency of 0.2 Hz. HTC-Waves, which we also demonstrate in vivo, critically depend on frequency facilitation of mossy fiber to CA3 synaptic transmission. They precisely follow the rhythm of the EC input, involve high-frequency firing (>100 Hz) of CA3 pyramidal neurons, and induce NMDA receptor-dependent CA1 LTP within a few seconds. We additionally show that HTC-Waves are rapidly boosted by the cognitive enhancer caffeine (5 µM), the stress hormone corticosterone (100 nM), the endogenous cannabinoid reuptake inhibitor AM404 (10 µM), the antidepressants amitriptyline, clomipramine, fluoxetine, citalopram, fluvoxamine, venlafaxine, tianeptine and tranylcypromine (all of them in the low micromolar range), and the mood stabilizer lithium (0.5 and 1 mM). In contrast, chronic stress, the antipsychotic haloperidol (10 µM), and the anxiolytic agent diazepam (0.1 and 1 µM) considerably weaken HTC-Waves. Taken together, our study provides the first experimental evidence that synchronous theta-rhythmical spiking of EC stellate cells, as occurring during EC theta oscillations, has the capacity to drive induction of CA1 LTP via the hippocampal trisynaptic pathway. Moreover, we present data pointing to a basic filter mechanism of the hippocampus regarding EC inputs and describe an in vitro real-time imaging assay to reveal

pharmacologically and environmentally induced alterations in the “input-output relationship” of the hippocampal trisynaptic circuit.

Disclosures: J. Stepan: None. **T. Fenzl:** None. **J. Dine:** None. **M. Eder:** None. **C.T. Wotjak:** None. **S.A. Polta:** None. **G. von Wolff:** None. **M.V. Schmidt:** None. **A. Uribe:** None. **F. Holsboer:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.13/F39

Topic: B.09. Network Interactions

Support: Intramural Research Program of the National Institute of Mental Health, NIH

Title: Finite-size effects identify feed-forward dynamics of neuronal avalanches

Authors: *S. YU, A. KLAUS, H. YANG, D. PLENZ;
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Abstract: Considerable effort is currently dedicated to identify mesoscopic brain dynamics by recording simultaneously from as many neurons as possible. For the mammalian cortex, high-density microelectrodes provide a restricted view into cortical dynamics that manifest at scales potentially much larger than the observation window. The finite-size effects of such ‘windowed’ recordings on characterizing the true neuronal dynamics of the much larger network are poorly understood. Here we study this problem by analyzing neuronal avalanches in ongoing local field potentials (LFPs) from awake macaque monkeys and neuronal modeling. We show that finite-size effects imposed by the recording window can be clearly identified and provide crucial insights into the feed-forward activity cascades that underlie scale-invariant neuronal avalanche dynamics. Spontaneous LFP activity (1 – 100 Hz) was recorded in superficial layers of pre-motor and prefrontal cortex in two awake macaque monkeys with chronically implanted, high-density 96-microelectrode arrays. Spatiotemporal LFP clusters were identified on the array and on compact sub-regions of the array corresponding to window size, N, ranging from $N = 10 – 95$. Clusters in each region organized as neuronal avalanches, i.e., the cluster size, s, followed a power law with exponent -1.5 up to N, beyond which the probability of cluster size declined steeply, referred to as the ‘finite-size effect’. Several additional observations were made. First, clusters with $s < N$ consisted mainly of non-repeating electrode activations. Thus, avalanches represent non-recurrent, feed-forward cascades. Second, clusters with $s > N$ reflect avalanches that activated all electrodes at least once in addition to electrode repeats. Thus, for $s > N$, the

avalanche size distribution captures the relatively small subset of avalanches that incorporate numerous repeats. Third, with increasing *N*, repeats increased for all cluster sizes $s < N$ and $s > N$. This reflects increasing dynamical re-activation of sites during avalanches with longer lifetimes and reduces the finite-size effect. These findings were confirmed in a model for N up to 625 that realized critical branching dynamics at the neuron level and provided a window of locally summed neuronal activity in a layer of LFP electrodes. In summary, the finite-size effect observed for neuronal avalanches reflects their nature of feed-forward cascades. We conclude that careful identification of finite-size effects allows for proper identification of the underlying dynamics despite windowing constraints when measuring from a subset of a neuronal population.

Disclosures: S. Yu: None. **A. Klaus:** None. **H. Yang:** None. **D. Plenz:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.14/F40

Topic: B.09. Network Interactions

Support: KAKENHI Grant 25430011 (to N.T.), 23680042, 24111551, 24650219 (to KFT)

Title: Dorsal and ventral hippocampal CA1 pyramidal neurons activate distinct brain area: Optogenetic investigation using mouse fMRI

Authors: *N. TAKATA[1], Y. KOMAKI[2], Y. SAKAI[4], K. YOSHIDA[3], M. XU[3], K. HIKISHIMA[2], H. OKANO[2], M. MIMURA[3], K. F. TANAKA[3];
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Abstract: Functional segmentation of the hippocampus along its dorsoventral axis has been proposed; dorsal hippocampus (DH) is involved in memory function while ventral hippocampus (VH) modulates emotional processes. Indeed, anatomical studies have revealed that extrinsic axonal projections from the field CA1 reaches over 50 areas and areal subdivisions and that these afferent fibers show distinct pattern depending on its origin (DH or VH). Gene expression research also demonstrated heterogenic spatial distribution patterns of some marker genes in the CA1 along the axis. Although these studies are suggestive of distinct function of DH and VH, physiological impact of DH- or VH-activity on other brain region would further deepen our understanding of functional differences of them. To this end, we combined functional MRI (fMRI) and a transgenic (Tg) mouse expressing a highly light-sensitive channelrhodopsin-2

mutant [ChR2(C128S)] in CA1 pyramidal neurons. Preliminary results show that illumination of DH or VH of an anesthetized Tg-mouse induced BOLD response in distinct area of the brain.

Disclosures: **N. Takata:** A. Employment/Salary (full or part-time):; Shionogi, Chugai Pharmaceutical, Meiji Seika Pharma, Mochida Pharmaceutical, Yoshitomiyakuhin, Dainippon Sumitomo Pharma. **Y. Komaki:** None. **Y. Sakai:** None. **K. Yoshida:** None. **M. Xu:** A. Employment/Salary (full or part-time):; Shionogi, Chugai Pharmaceutical, Meiji Seika Pharma, Mochida Pharmaceutical, Yoshitomiyakuhin, Dainippon Sumitomo Pharma. **K. Hikishima:** None. **H. Okano:** None. **M. Mimura:** None. **K.F. Tanaka:** A. Employment/Salary (full or part-time):; Shionogi Inc., Chugai Pharmaceutical, Meiji Seika Pharma, Mochida Pharmaceutical, Yoshitomiyakuhin, Dainippon Sumitomo Pharma.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.15/F41

Topic: B.09. Network Interactions

Support: UConn startups to MV

Title: Neuronal encoding and the site of action potential initiation

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Abstract: Populations of neocortical neurons can encode rapidly changing signals and respond to subtle inputs fast, within few ms. Theoretical analysis of single-compartment models attributed such coding properties to action potential (AP) generators with fast onset dynamics. These models, however, do not take into account electrical compartmentalization of neurons. In neocortical neurons, membrane potential produced fluctuations produced by incoming inputs and the APs are usually recorded in the soma, while initiation of the APs takes place in the axon initial segment (AIS), at some distance from the soma and recording site.
Here we ask whether encoding properties of neurons depend on the site of AP initiation. Using whole-cell recordings in slices from rat neocortex we show that populations of layer 5 pyramidal neurons can encode fast input changes by phase-locking their firing to frequencies up to ~500-600 Hz. Using fast optical imaging with voltage-sensitive dyes we show, in accordance with prior reports, that APs in these neurons are initiated in the AIS, 50-55 μm from the soma. For

computer simulations, we constructed families of multicompartmental neuron models with systematically shifted site of AP initiation along the axon: in the axon hillock, proximal or distal axon initial segment and 1st to 4th node of Ranvier. In the models with Hodgkin-Huxley type sodium channels the encoding of high frequencies was improved when AP initiation site was shifted from the soma to the distal AIS or the first node of Ranvier, but deteriorated with further shift to the 2nd - 4th nodes. However, even with the optimal location of AP initiation site at 50-120 μm from the soma, the encoding of high frequencies in HH type models was significantly inferior to that in neurons. In the second and third families of models a small fraction (10%) of sodium channels with threshold-like activation or cooperative activation was added at the site of AP initiation. All other sodium channels in these models had HH type kinetics. Models with a fraction of threshold channels at the initiation site encoded high frequencies better than the neurons. Encoding in the models with a fraction of cooperative channels at the initiation site was better than in HH type models, but less robust than in models with a fraction of threshold channels. In contrast to HH type models, encoding of high frequencies in the models with a fraction of threshold or cooperative channels was optimal when spikes were initiated in the soma or axon hillock, but was slightly attenuated with the shift of AP initiation site away from the soma, most probably due to passive filtering of membrane potential fluctuations on their way to the AP initiation site.

Disclosures: M.A. Volgushev: None. **V. Ilin:** None. **E. Nikitin:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.16/F42

Topic: B.09. Network Interactions

Support: NIH R01 DC006013

Title: Effects of isoflurane on cortical UP state activity in brain slices

Authors: *H. HENTSCHKE[1], A. RAZ[2], B. M. KRAUSE[3], S. M. GRADY[2], M. I. BANKS[2]; [1]Anaesthesiol., Univ. Tuebingen, Tuebingen, Germany; [2]Dept. of Anesthesiol., [3]Neurosci. Training Program, Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Loss of consciousness under volatile anesthetics is likely mediated via direct actions on the cortico-thalamic network. We have detailed knowledge of the molecular targets of these agents, and end effects on cortically mediated behaviors are similarly well described, but how these agents alter activity at the network level is unclear. Cortical UP states

represent all-or-none, synchronous, propagating bouts of network activity. UP states and the aroused cortical state share key characteristics, and evidence suggests parallel continua from bistable UP/DOWN to desynchronized network activity and from the unconscious to the aroused behavioral state. Thus, modulation of UP states by anesthetics may shed light on the mechanisms of these agents' hypnotic effects.
Methods: Acute auditory thalamocortical (TC) brain slices were prepared from 4-13 wk mice. Local field potentials (LFPs) and multiunit activity (MUA) were recorded using silicon multielectrodes (16 shanks, 100μm spacing, 1 site/shank) oriented either perpendicular to the pial surface or rostro-caudally in layer 5 of auditory cortex. TC and corticocortical (CC) afferents were stimulated using bipolar tungsten electrodes. Cocultures of two cortical slices were prepared from P3 mice and used after 2 wk in vitro, at which time the pair of slices had established mutual synaptic connections. LFPs and MUA were recorded with glass electrodes (one in each slice).
Results: Both acute and cultured slices dwelled primarily in DOWN states, with occasional (acute) or frequent (cultured) spontaneous UP state transitions. Afferent stimulation reliably evoked UP states in all slices. In acute slices, thresholds were lower for TC versus CC stimulation, but for both stimuli UP states originated in layer 5 and spread to supragranular layers. UP state activity propagated several millimeters along the layers at a rate dependent on stimulus efficacy (range 8 - 42 μm/ms). In cultures, spontaneous or stimulated UP states originating in one slice triggered UP states in the other with lags of 10 - 300 ms. Isoflurane (0.5 - 1%) increased thresholds for evoking UP states and decreased their propagation velocity (acute), shortened stimulated and spontaneous UP states and depressed the frequency of occurrence of spontaneous UP states (cultured) and decreased trial-by-trial variability of LFP and MUA activity (both).
Conclusions: Isoflurane suppresses UP state activity in cortical networks, and may increase reproducibility of stimulated activity by suppressing background activity outside UP states.

Disclosures: H. Hentschke: None. **A. Raz:** None. **B.M. Krause:** None. **S.M. Grady:** None. **M.I. Banks:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.17/F43

Topic: B.09. Network Interactions

Support: DBT

DAE

EU-India Grid

NIGMS/SBCNY

NCBS

Title: Reliability of network behaviour depends on excitation/inhibition balance and distribution of synaptic weight

Authors: *S. RAY, U. S. BHALLA;
NCBS, Bangalore, India

Abstract: We investigated the behaviour of a neuronal network model of thalamus to cortical layer 4 circuit with varying number of inhibitory neurons. In addition, we also studied how the distribution of synaptic weights affects the network behaviour.
We ported components from a published model (Traub et al 2005) to the Multiscale Object-Oriented Simulation Environment (MOOSE) to construct a network of excitatory spiny stellate (SS) cells of cortical layer 4, inhibitory deep basket (DB) and deep LTS (DLTS) cells. This network received inputs from a set of thalamocortical relay (TCR) cells stimulated electrically. The cell populations were connected via GABAA or AMPA and NMDA synapses. We simulated multiple instances of this network with varying number of deep basket cells while keeping the total synaptic conductance on each postsynaptic cell constant. The synaptic strengths were either constant between a pair of cell types or was normally distributed. Later we simulated the network with lognormal distribution of the AMPA conductance.
The SS population in the network displayed spontaneous synchronized spiking. As the number of inhibitory cells increased, the period of this synchronized spiking became more variable and smaller fractions of SS cells participated in the synchronized events. In these simulations the stimulus via TCR cells had little effect on the SS population. On the other hand, when the AMPA conductances between each pair of cell types were lognormally distributed, such that there were only a few strong synapses with many weak synapses, the spontaneous population spiking was eliminated and there was synchronized population spiking in response to the stimulus.
Inhibitory interneurons, though few in number, have strong effect on network behaviour. We find that the balance of excitation and inhibition is crucial but so is the synchrony of inhibition as tested by scaling the number of basket cells while keeping the total synaptic input constant. Moreover the distribution of synaptic weights is important in eliciting response to stimulus. A lognormal distribution is most effective for this. Our work shows that detailed experimental investigation of these aspects will be vital in understanding cortical processing.

Disclosures: S. Ray: None. **U.S. Bhalla:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.18/F44

Topic: B.09. Network Interactions

Title: Exploration of the input-output relation of granule cells in the dentate gyrus

Authors: A. D. MADAR[1], L. A. EWELL[2], *M. V. JONES[1];
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Abstract: Although extensive work has been done to understand hippocampal processing, a clear mechanistic view of how information is processed at the very first stage, inside the Dentate Gyrus (DG), is still elusive. To rigorously explore the computational role of the DG requires knowledge of both the input and output patterns. It is difficult to obtain this information in vivo, whereas the accessibility of the in vitro brain slice provides an ideal, yet largely unexploited, platform. Input to the DG arrives via the perforant path from entorhinal cortex (EC), whereas Granule Cells (GCs) constitute the output to the CA3 region. To explore the input-output relation of the DG, we performed whole-cell recordings of GCs in acute slices of mouse brain, while stimulating the lateral perforant path (LPP) or the medial perforant path (MPP) arising from the lateral or medial entorhinal cortex (LEC or MEC) respectively. Poisson stimulus trains (100 µs pulses, 0.1-10 mA) were delivered through a <5 µm theta pipette with a mean rate of 10 Hz, a frequency shown to preferentially drive spike and burst generation in GCs (Ewell & Jones, 2010). Alternating between voltage- and current-clamp, we recorded postsynaptic currents (i.e., synaptic input) and membrane potential fluctuations (i.e., spiking output) during the presentation of 10 different stimuli trains. This way, it was possible to measure how incoming signals are integrated by GCs.
First we characterized what kind of inputs make GCs fire by averaging the LPP or MPP stimulus trains that immediately preceded each spike. These Spike-Triggered Averages (STA) showed that different GCs were responsive to different spatial and temporal patterns, some being more sensitive to LPP inputs, others to MPP or equally responsive to both. To further understand how input patterns are transformed into spikes, we asked what patterns of postsynaptic currents lead to action potentials. To do this we performed STA on the current traces assumed to be good estimates of the current fluctuations when the membrane potential was recorded. Surprisingly, 5/7 GCs showed that the EPSC preceding a spike was on average closely followed by a large secondary EPSC. Indeed, looking at individual spike-triggered current waveforms, a large post-

spike EPSC was often present. In all GCs, Principal Component Analysis on the spike-triggered ensemble of current waveforms detected multiple clusters, suggesting that GCs spike in response to a variety of current waveform shapes, some of which have a late EPSC. The source of the late EPSC is not clear but could be explained by feedback excitation via mossy cells or by back-propagating action potentials unmasking NMDA currents.

Disclosures: A.D. Madar: None. **L.A. Ewell:** None. **M.V. Jones:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.19/F45

Topic: B.09. Network Interactions

Support: NIH/NICHD 1R01HD062577-01

Title: The GABAB modulation affects recurrent horizontal flow of activity & local synchrony in an 'Up-State' model of cortical functioning

Authors: *R. G. PORT[1], M. F. MCMULLEN[2], G. C. CARLSON[2];
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Abstract: GABAergic compounds have recently been shown efficacious in the partial treatment of Autism Spectrum Disorders and related neurodevelopmental diseases. A possible substrate for these treatments could be the reduction of local circuit hyperconnectivity, and recovery of network synchrony. As the first step in a series of experiments, we have modeled cortical functioning to thalamic input using a media that promotes the creation of both spontaneous, and thalamic stimulation evoked recurrent cortical network activity. Recordings of network activity were acquired using both voltage sensitive dye imaging of auditory cortex and electrodes placed in supragranular/granular lamina of auditory cortex for local field potentials. Data was then analyzed using Igor and the Matlab toolbox Fieldtrip to examine spread and frequency coherence profiles of circuit activity. After 30 minutes of baclofen exposure, both the propagation of recurrent network activity, and measures of local synchrony (gamma-band activity) are reduced. This work is part of an ongoing project studying neural activity abnormalities in early auditory processing for Autism Spectrum Disorders. This work was supported by NIH/NICHD 1R01HD062577-01.

Disclosures: R.G. Port: None. **M.F. McMullen:** None. **G.C. Carlson:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.20/F46

Topic: B.09. Network Interactions

Support: NIH NS076706

Title: Regulation of dynamic range in CA1 pyramidal neurons by feedforward inhibition and background noise

Authors: *A. KHUBIEH[1,2], S. RATTE[1], S. A. PRESCOTT[1,3];
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Abstract: Regulating the gain of a neuron's input-output curve is crucial for insuring that this neuron can encode over an appropriate range of stimulus intensities. It has been argued that pyramidal neurons in the CA1 region of hippocampus have very steep input-output curves in response to Schaffer collateral stimulation, and that it is by activating more neurons that the network as a whole (rather than any single neuron) can encode the stimulus intensity. The balance of signal-dependent excitation and feedforward inhibition (FFI) plays an important role in that process. However, other studies have shown that background synaptic activity is also important for gain control. We hypothesized that background activity would reduce input-output gain, broadening the encoding range.
To test our hypothesis, we recorded CA1 pyramidal neurons from a rat hippocampal slice preparation. We applied stimuli in one of two ways: by stimulating the Schaffer collaterals or by injecting virtual synaptic waveforms directly into the recorded neuron using dynamic clamp. In both experiments, neurons were subjected to background activity simulated using two Ornstein-Uhlenbeck processes, each for excitatory and inhibitory conductance, and applied through dynamic clamp. We then used computer simulations and mathematical analysis to test different values of input synchrony and latency to inhibition to explore how these parameters impact the input range in this modified context. In each experiment, we measured the probability of spiking induced by the stimulation for different input strengths.
Comparing conditions with and without background activity, we found that neurons displayed a broader dynamic range under the realistic conditions (i.e. with background activity). By testing different background activity parameters, we found that shunting is responsible for a depolarized spiking threshold whereas increasing noise is responsible for decreasing the input-output gain.

Finally, simulations showed that diverse combinations of parameters of FFI adjust the input range in different ways.
Our results suggest individual CA1 pyramidal neurons can encode a broad range of stimulus intensities when they operate under noisy conditions. But because the response of any one neuron is noisy, the output from a set of neurons must be considered in order to decode the intensity of any one stimulus. FFI still contributes to regulating the dynamic range.

Disclosures: A. Khubieh: None. **S. Ratte:** None. **S.A. Prescott:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.21/G1

Topic: B.09. Network Interactions

Title: Traveling alpha waves in the human electrocorticogram

Authors: *H. ZHANG[1], A. D. SHARAN[3], M. R. SPERLING[3], J. JACOBS[2];
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Abstract: Over decades of research, brain oscillations have been shown to play a critical role in various aspects of human brain function. In particular, oscillations in the theta and alpha bands (4-16 Hz) have been shown to vary in amplitude in relation to neural processes related to memory and cognition. In addition to these amplitude changes, research in animals has shown that brain oscillations exhibit complex patterns of phase changes across space, including traveling and spiral waves across the cortex and hippocampus. However, little evidence for similar patterns in humans has been found. We developed a new method to detect spatial patterns of brain oscillations and used this approach to probe human brain oscillations in electrocorticographic recordings from epilepsy patients performing a memory task. We found spatial patterns of synchronized oscillations in a range of frequencies, including the theta, alpha and beta bands. These patterns included both traveling and standing waves, as well as complex hybrid patterns. We analyzed the direction of these traveling waves and found that alpha waves during the task emanate outward from the temporal lobe and travel to the frontal and occipital lobes. Our results support the hypothesis that traveling waves aid in information flow and help to coordinate large-scale brain networks. We discuss the possible role of alpha oscillations in gating top-down and bottom-up information flow during human memory processing.

Disclosures: H. Zhang: None. **A.D. Sharan:** None. **M.R. Sperling:** None. **J. Jacobs:** None.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.22/G2

Topic: B.09. Network Interactions

Support: NIH -R01-NS-078789

Title: Repeated nerve block using charge balanced direct current through high surface area electrodes

Authors: *T. L. VRABEC[1], M. FRANKE[1], J. S. WAINRIGHT[2], N. BHADRA[1], N. BHADRA[1], K. L. KILGORE[3,1,4];
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Abstract: There are many neurological diseases that result in undesirable neural activity such as spasticity, movement disorders, and chronic pain of peripheral origin. Existing treatment options such as pharmaceuticals are insufficiently effective, and can result in significant side effects. A fast acting, reversible electrical conduction block is an advantageous option for meeting these needs. KiloHertz Frequency Alternating Current (KHFAC) has been shown to provide a fast acting nerve block that is quickly reversed when the KHFAC is halted. One drawback of KHFAC is that when it is first initiated, a brief burst of action potentials occurs which can cause undesirable effects. This "onset response" can be eliminated by briefly applying direct current (DC) block to electrodes flanking the KHFAC electrode. One obstacle to this method is the occurrence of neural damage from the DC due to electrochemical reactions at the electrode-tissue interface. An electrode design that can deliver DC block while not producing the electrochemical products might prevent nerve damage. The production of electrochemical products can be measured by cyclic voltammetry to determine the water window, which is the region where neither oxidation nor reductions occurs. There are several types of electrode coatings (e.g. platinum black, iridium oxide) that can increase the capacitance of the electrode, allowing more charge to be delivered while remaining within the water window. Monopolar nerve cuff electrodes were fabricated using platinum foil and then platinized in chloroplatinic acid solution to create platinum black coatings with different charge capacities. Typical values are in the range of 18mC to 21 mC. These electrodes were tested in acute experiments on the rat sciatic nerve using current-controlled, charge-balanced DC (CBDC) waveforms. The DC waveforms were repeatedly applied for 200 cycles of DC delivery or until nerve conduction was

compromised. Each cycle consisted of a 4 second ramp down, a 7 second plateau, and a 2 second ramp up for a total of 2600 sec of DC delivery over 200 cycles. The reduction of nerve conduction was measured by stimulating the nerve proximal and distal to the blocking site and comparing the force output of the muscle. The platinum black electrodes were capable of delivering the full 200 cycles (3200 mC) without any reduction in nerve conduction.

Disclosures: T.L. Vrabec: None. **M. Franke:** None. **J.S. Wainright:** None. **N. Bhadra:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuros Medical Inc. F. Consulting Fees (e.g., advisory boards); Neuros Medical Inc. **N. Bhadra:** None. **K.L. Kilgore:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuros Medical Inc. F. Consulting Fees (e.g., advisory boards); Neuros Medical Inc.

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.23/G3

Topic: B.09. Network Interactions

Support: NIH-R01-NS-078789

Title: Combined no-onset KHFAC+DC nerve block without nerve damage

Authors: *M. FRANKE[1], T. L. VRABEC[1], J. L. WAINRIGHT[1], N. BHADRA[1], N. BHADRA[1], K. L. KILGORE[2,3,1];
[1]Case Western Reserve Univ., Cleveland, OH; [2]Metro Hlth. Med. Ctr., Cleveland, OH; [3]Louis Stokes VA Med. Ctr., Cleveland, OH

Abstract: Kilo-Hertz frequency alternating current waveforms (KHFAC) have been shown to provide rapid, complete and fully reversible nerve block for durations up to 60 minutes. Chronic preclinical and pivotal clinical studies have shown efficacy and first safety data for an implementation of KHFAC block in applications for pain and obesity. However, one major side effect of this electric block has not been addressed so far: whenever KHFAC block is initiated, there is an intense transient activation of nerve fibers, termed the onset-response. The onset-response can last for seconds and has the potential to cause discomfort or pain to the patient, limiting potential clinical applications.
A ramped direct current (DC) waveform can produce nerve block without an onset-response. A brief (less than ten seconds) DC block through a separate electrode can prevent transmission of

the onset response produced by the KHFAC, while continued block is then maintained by KHFAC waveforms. A previous study showed that though this strategy worked, it resulted in rapid permanent nerve conduction failure if the injected DC charge was un-balanced. Change in nerve conduction occurred within 30 sec and complete permanent nerve conduction failure occurred within 60 sec of cumulative DC delivery. High surface area electrodes and the use of charge-balanced DC (CBDC) waveforms can solve this problem by ensuring that the electrode voltage during the CBDC remains within the water window (potential difference of -0.6 V to +0.9 V vs Ag/AgCl).
This study combined ramped CBDC and KHFAC waveforms to achieve an electric nerve block. Two separate block electrodes were acutely implanted on the rat sciatic nerve. The KHFAC electrode was located proximal to the high-surface area CBDC electrode. The block waveforms were initiated sequentially. After a full sciatic nerve (motor) block was achieved with the CBDC waveform, KHFAC block was initiated. While the sciatic nerve block was maintained by KHFAC, DC was charge balanced and then terminated. Nerve conduction integrity was monitored by comparing muscle forces produced by supra-threshold activation from an electrode proximal to both block electrodes with that from an electrode distal to both block electrodes. The results showed consistent “no-onset block” by using this combined nerve block method. Repeated CBDC block of durations up to 13 seconds each (plateau current range: -1.2 to -2.2 mA), followed by KHFAC block for up to 120 seconds (voltage range: 8 to 10 Vpp, 20 kHz) were applied for up to 30 cycles. We were able to show that CBDC block did not adversely affect nerve conduction with over 300 seconds of cumulative CBDC block time.

Disclosures: M. Franke: None. **T.L. Vrabec:** None. **J.L. Wainright:** None. **N. Bhadra:** None. **N. Bhadra:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuros Medical Inc.. F. Consulting Fees (e.g., advisory boards); Neuros Medical Inc. **K.L. Kilgore:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuros Medical Inc.. F. Consulting Fees (e.g., advisory boards); Neuros Medical Inc..

Poster

520. **Network Interactions: Signal Propagation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 520.24/G4

Topic: B.09. Network Interactions

Support: JFRC visiting scientist program

NINDS intramural research program

Title: Clustered thalamocortical input onto layer 5 pyramidal neurons detected using quantitative large-scale array tomography

Authors: *J.-C. RAH[1,2], E. BAS[1], J. COLONELL[1], Y. MISHCHENKO[3], B. KARSH[1], R. FETTER[1], E. MYERS[1], D. CHKLOVSKII[1], K. SVOBODA[1], T. HARRIS[1], J. ISAAC[2]; [1]Janelia Farm Res. Campus, Ashburn, VA; [2]Developmental Synaptic Plasticity Section, Natl. Inst. of Neurolog. Disorders and Stroke, Natl. Inst. of Hlth., Bethesda, MD; [3]Dept. of Engineering, Toros Univ., Yenisehir, Turkey

Abstract: The subcellular locations of synapses on pyramidal neurons strongly influences dendritic integration and synaptic plasticity. Despite this, there is little quantitative data on spatial distributions of specific types of synaptic input. Here we use array tomography (AT), a high-resolution optical microscopy method, to examine thalamocortical (TC) input onto layer 5 pyramidal neurons. We verify the ability of AT to identify synapses by means of parallel electron microscopic analysis. We then use large-scale AT to measure TC synapse distribution in a 1.00 x 0.83 x 0.21 mm^3 volume of mouse somatosensory cortex. We found that TC synapses primarily target basal dendrites in layer 5, but also make a considerable input to proximal apical dendrites in L4, consistent with previous work. However, we also found that TC inputs are biased towards certain branches and, within branches, synapses show significant clustering with an excess of TC synapse nearest neighbors within 5-15 um compared to a random distribution. Thus, we show that AT is a sensitive and quantitative method to map specific types of synaptic input on the dendrites of entire neurons. We anticipate that this technique will be of wide utility for mapping functionally-relevant anatomical connectivity in neural circuits.

Disclosures: J. Rah: None. **E. Bas:** None. **J. Colonell:** None. **Y. Mishchenko:** None. **B. Karsh:** None. **R. Fetter:** None. **E. Myers:** None. **D. Chklovskii:** None. **K. Svoboda:** None. **T. Harris:** None. **J. Isaac:** None.**Poster**

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.01/G5

Topic: B.11. Glia-Neuron Interactions

Support: FONDECYT1110571

DID-UACh

Title: Neuronal antioxidant defense contribution of astrocytes-derived exosomes containing ascorbic acid

Authors: *P. TRONCOSO ESCUDERO[1], F. BELTRÁN[1], F. COURT[2], M. CASTRO GALLASTEGUI[1];
[1]Univ. Austral De Chile, Valdivia, Chile; [2]Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: Astrocytes have long been considered as just providing trophic support for neurons in the central nervous system. However, from few decades ago, several studies have demonstrated astrocytic importance in many functions such as neurotransmission, redox balance, metabolite and electrolyte homeostasis, among others. Ascorbic acid is a powerful antioxidant which is concentrated in brain. During synaptic activity ascorbic acid is released by astrocytes and taken up by neuronal cells. In neurons ascorbic acid is utilized to maintain redox balance and to modulate neuronal metabolism. Molecular and cellular basis of ascorbic release is not fully understood. In this work we studied ascorbic acid release from astrocytes through exosomes, extracellular microvesicles and hemichannels. Microvesicles and exosomes were purified from supernatant of primary cultures of cortical astroglial cells and human astrocytes cell line by centrifugation and ultracentrifugation, respectively. HPLC-detected ascorbic acid was released through exosomes and microvesicles in astrocytes. Also, the application of LaCl3, a hemichannel inhibitor, decreased ascorbic acid efflux suggesting an important role of connexins in ascorbic acid release from astrocytes. Finally, we demonstrated that ascorbic acid-containing exosomes derived from astrocytes is able to modulates the expression pattern of proteins related to ascorbic acid recycling and homeostasis in neuronal cells. Mechanisms of ascorbic acid exportation form glial to neuronal cells are indispensable to maintain the correct neuronal function. Impaired ascorbic acid release through these mechanisms could be related to redox and metabolic failures described in neurodegenerative diseases. FONDECYT1110571. DID-UACh.

Disclosures: P. Troncoso Escudero: None. **F. Beltrán:** None. **F. Court:** None. **M. Castro Gallastegui:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.02/G6

Topic: B.11. Glia-Neuron Interactions

Title: Noradrenaline attenuates hydrogen peroxide-induced cell death of astrocyte through the increase in the level of intracellular glutathione

Authors: *H. KADOI, Y. YOSHIOKA, A. YAMAMURO, Y. ISHIMARU, S. MAEDA; Setsunan Univ., Hirakata, Japan

Abstract: Noradrenaline (NA) has been known to modulate the functions of astrocytes. However, the effect of NA on the antioxidative capacity of astrocytes has been poorly investigated. In this study, we investigated the effect of NA on hydrogen peroxide (H_2O_2) toxicity in human astrocytoma U-251 MG cells. Cell viability was estimated by a colorimetric MTT assay, and intracellular glutathione (GSH) level was determined by DTNB recycling assay. Treatment of U-251 MG cells with H_2O_2 for 24 h induced cell death in a concentration-dependent manner. Pretreatment with NA (1-100 μM) for 24 h concentration dependently attenuated the H_2O_2-induced cell death. The cytoprotective effect of NA was inhibited by a β-adrenoreceptor antagonist propranolol, and pretreatment with a β-adrenoreceptor agonist isoproterenol (30-100 μM) attenuated the H_2O_2-induced cell death. Treatment with NA (1-100 μM) for 24 h concentration-dependently increased intracellular GSH level. In the presence of DL-buthionine-[S,R]-sulfoximine, a GSH synthesis inhibitor, the increase of intracellular GSH and the cytoprotection by NA were abolished. The NA-induced increase in intracellular GSH was inhibited by propranolol. Western blot analysis revealed that NA increased the level of glutamate-cysteine ligase (GCL), the rate-limiting enzyme in GSH synthesis. The induction of GCL protein by NA was inhibited by propranolol. The cytoprotective effect, the increase of intracellular GSH and the induction of GCL protein by NA were inhibited by a β_3-adrenoreceptor antagonist SR59230A, but not by a β_1-adrenoreceptor antagonist atenolol and a β_2-adrenoreceptor antagonist butoxamine. These results indicate that NA protects U-251 MG cells against H_2O_2-induced death through the increase in the level of intracellular GSH, and that NA increases the intracellular GSH level through the induction of GCL protein via β_3-adrenoreceptor stimulation.

Disclosures: H. Kadoi: None. **Y. Yoshioka:** None. **A. Yamamuro:** None. **Y. Ishimaru:** None. **S. Maeda:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.03/G7

Topic: B.11. Glia-Neuron Interactions

Title: Noradrenaline protects neurons from hydrogen peroxide-induced death by increasing the supply of glutathione from astrocytes

Authors: S. MAEDA[1], A. YAMAMURO[1], Y. ISHIMARU[1], *Y. AGO[2], Y. YOSHIOKA[1]; [1]Setsunan Univ., Hirakata, Japan; [2]Osaka Univ., Suita/Osaka, Japan

Abstract: Hydrogen peroxide (H_2O_2) has been implicated in a variety of neurodegenerative disorders, such as Parkinson's disease. Astrocytes express many types of functional neurotransmitter receptors, and play a significant role for neuronal survival by supplying antioxidants such as glutathione (GSH) to neurons. Recently, we found that noradrenaline (NA) increased GSH in astrocytes. Thus, NA may protect neurons from oxidative stress-induced death by increasing the supply of GSH from astrocytes to neurons. In this study, we investigated the neuroprotective effect of NA by using the co-culture system of human neuroblastoma SH-SY5Y cells and human astrocytoma U-251 MG cells. We established SH-SY5Y cells overexpressed green fluorescence protein (GFP), and the cell viability was determined based on the morphology of GFP-positive cells under a fluorescence microscope. SH-SY5Y cells were co-cultured with U-251 MG cells as a mixture (mixed co-culture) or separately using culture insert (separated co-culture). To investigate the intracellular GSH level in SH-SY5Y cells in mixed co-culture, the cells were stained with reduced GSH-reactive probe monochlorobimane, and were analyzed by Cellomics ArrayScan. Pretreatment with NA (10-30 μM) for 24 h protected SH-SY5Y cells from H_2O_2-induced death in mixed co-culture, but not in single culture. NA increased the intracellular GSH levels of SH-SY5Y cells in mixed co-culture, but not in single culture. DL-buthionine-[S,R]-sulfoximine, a GSH synthesis inhibitor, negated the cytoprotective effect of NA in mixed co-culture. In separated co-culture with U-251 MG cells, NA failed to increase the intracellular GSH levels in SH-SY5Y cells and did not protect the cells from H_2O_2-induced cell death. These results indicate that NA protects SH-SY5Y cells from H_2O_2-induced death by increasing the supply of GSH from U-251 MG cells, and that the supply of GSH requires direct cell-cell contact between SH-SY5Y cells and U-251 MG cells.

Disclosures: S. Maeda: None. **Y. Ago:** None. **Y. Ishimaru:** None. **A. Yamamuro:** None. **Y. Yoshioka:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.04/G8

Topic: B.11. Glia-Neuron Interactions

Support: ETSU Major RDC 12-018M

Ronald McNair Post Baccalaureate Achievement Program - TRIO

Title: *In vitro* characterization of glial-derived neurotrophic factor upregulation, receptor internalization, and antioxidant adaptations to oxidative stress

Authors: *C. E. BOND, J. CRANMORE, S. FREGOSO, G. L. WRIGHT, D. B. HOOVER; Dept. of Biomed. Sci., Eastern Tennessee State Univ., Johnson City, TN

Abstract: Reactive astrogliosis, characterized by glial inflammation and proliferation, is the hallmark feature of both acute and chronic central nervous system damage in a majority of degenerative pathologies, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), the three most common degenerative brain disorders. Astroglia control the supply of metabolites through the blood-brain barrier, modulate signal transmission at the synapse, activate microglia in response to damage, and release trophic factors, such as glial-derived neurotrophic factor (GDNF) and neurturin (NTN), essential for neuronal proliferation and survival, during development and throughout adulthood. Although early experiments demonstrated that GDNF & NTN can rescue degenerating dopamine neurons in animal models of Parkinson's disease and enhance survival of cultured cholinergic motor neurons, results in human clinical trials have been disappointing.

Few studies have focused on the effects of these trophic factors on the cells that produce them, despite evidence that GDNF exerts its effect on neurons indirectly by stimulating growth factor release from intermediary glial cells, and confirmation that astroglia express cell surface receptors for both NTN & GDNF. In addition, despite the importance of stress-induced glial dysregulation in perpetuating degenerative processes, little is known about the cellular actions of these trophic factors under stress conditions. To clarify these mechanisms, this study examines oxidative stress-induced changes in GDNF-family gene expression, mitochondrial function and biochemical responses in primary cultures of astroglia from wild-type C57BL/6 and a NTN knockout mouse model for comparative studies. Main Findings: Quantitative real-time PCR revealed dose-dependent increases in GDNF and GFRα2 gene expression after H_2O_2 exposure, accompanied by changes in protein levels as determined by Western blotting and immunostaining. Glial cells treated with NTN also increased surface expression of GFRα2. We observed alterations in glial mitochondrial bioenergetic profiles, changes in intracellular antioxidant balance and altered mitochondrial inner membrane protein 8A (Timm8A) staining, in response to oxidative stress. Since GDNF & NTN are well-documented neuronal reparative factors, these studies hold potential for enhanced understanding and manipulation of glial-neuronal interactions in therapeutic strategies for neurodegenerative diseases.

Disclosures: C.E. Bond: None. **J. Cranmore:** None. **S. Fregoso:** None. **G.L. Wright:** None. **D.B. Hoover:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.05/G9

Topic: B.11. Glia-Neuron Interactions

Support: NIA/NIH R01AG033720

Title: Age-dependent contribution of nitric oxide synthase to ischemic white matter injury

Authors: *S. BALTAN, J. ZALESKI, A. BACHLEDA, A. RUNKLE, S. BRUNET;
Dept. of Neurosciences, Cleveland Clin., Cleveland, OH

Abstract: Human brain comprises gray and white matter (WM) in equal volumes, which means that injury sustained after a stroke involves both. In addition, stroke risk increases considerably with age. Ischemic injury is a result of converging ionic, excitotoxic and oxidative stress pathways in young WM. In older WM the injury is mediated by enhanced excitotoxicity due to an earlier and more robust glutamate release. Recently we showed that even after maximal glutamate accumulation, preserving mitochondrial dynamics and ATP levels promote axon function recovery in young and old mouse optic nerves (MONs) suggesting that mitochondria related oxidative stress is a common component of ischemic injury in WM across irrespective of age. In this study we investigated whether blocking nitric oxide synthase (NOS) activity before or after a period of oxygen glucose deprivation (OGD) promoted functional recovery in an age-dependent manner.

Isolated MONs from young and old (1 and12 month) Swiss Webster (SW) or C57BL/6 (BL6) mice were used to ascertain quantitative measurements of WM function and structure. To support a biological basis for NOS inhibitor nitro-L-arginine methyl ester (LNAME) action on axon function, we evaluated the expression and localization of brain NOS (bNOS) using immunohistochemistry in combination with confocal microscopy. The expression of bNOS co-localized with GFAP (+) astrocyte nuclei, cytoplasm, end-feet as well as NF200 (+) axons. The pattern of bNOS expression paralleled astrocyte morphology with age and became more punctate in appearance. Evoked compound action potentials (CAPs) recovered to 21.8 ± 2.8% (n=18) after 60 min of oxygen glucose deprivation (OGD) in young BL6 MONs. Pretreatment of MONs with L-NAME (200 µM) promoted CAP recovery to 69.4 ± 11.3 % (n=8) compared to OGD while CAPs recovered to 39.9 ± 4.4% (n=11) when LNAME was applied after the end of OGD. Pretreatment of MONs from 1 or 12 month old SW with LNAME improved CAP recovery to 49.6 ± 3.7% (n=8, vs OGD 21.3 ± 3.7%, n=12) or to 51.1 ± 9.3% (n=7, vs OGD 5.7 ±1.7%, n=8) respectively. LNAME application after the end of OGD failed to promote aging axon function (8.8 ± 3.5%, n=6). Changes in NOS activity may help reveal age-dependent oxidative injury mechanisms involved in ischemic WM injury.

Disclosures: S. Baltan: None. **J. Zaleski:** None. **A. Bachleda:** None. **A. Runkle:** None. **S. Brunet:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.06/G10

Topic: B.11. Glia-Neuron Interactions

Support: NIH-NINDS-R01-NS065201

NIH-NIGMS-SC1-GM088019

NIH-NIMHD-G12-MD007583

Title: Pathways for physiological accumulation of polyamines in astrocytes

Authors: *Y. RIVERA[1], M. INYUSHIN[1], Y. V. KUCHERYAVYKH[1], M. SALA-RABANAL[4], L. Y. KUCHERYAVYKH[2], J. BENEDIKT[1], A. ZAYAS-SANTIAGO[3], R. W. VEH[5], C. G. NICHOLS[4], M. J. EATON[2], S. N. SKATCHKOV[1];
[1]Physiol., [2]Biochem., [3]Pathology and Lab. Med., Univ. Central Caribe, Bayamon, Puerto Rico; [4]Cell Biol., Washington Univ. Sch. of Med., Saint Louis, MO; [5]Charite, Berlin, Germany

Abstract: The polyamines (PAs), spermine (SPM), spermidine (SPD) and putrescine (PUT) are small aliphatic amines that regulate multiple biological processes and are ubiquitous in all organisms and essential for life. The endogenous PAs, specifically SPD and SPM, strongly affect various ion channels and receptors in the brain and exert neuroprotective, antidepressant, antioxidant and further effects. PAs of the central nervous system are predominantly localized in glial cells, while neurons only contain low SPM concentrations (Laube and Veh, 1997; Skatchkov et al., 2000). However, glial cells lack the enzymes for SPD/SPM synthesis (Madai et al., 2012; Kraus et al., 2006; 2007; our preliminary data), and very little is known about the mechanisms of transport of SPD and SPM in these cells. The purpose of the present study is to investigate the possible pathways for PAs transport through the glial membrane. First, we showed that exogenously applied spermine can be taken up by and accumulated in astrocytes. We applied exogenous biotinylated-SPM (B-SPM) to astrocyte cultures in the presence of physiologically high concentrations of divalents (extracellular calcium and magnesium 2 mM). We visualized the SPM that was taken up into the cells using rhodamine conjugated with avidin and confocal microscopy. A dose dependent (20, 40, and 80 μM) uptake of B-SPM into astrocytes was observed. There are several potential pathways for SPM uptake by astrocytes. Our

results now point to two likely major pathways for SPM/SPD uptake into astrocytes. The first likely pathway for PA transport is through connexin hemichannels. We found that SPM may permeate the astrocytic membrane through Cx43 hemichannels because blockers of Cx43 hemichannels as well as siRNA against Cx43 abolished SPM, SPD and PUT currents. The second likely pathway for SPM/SPD uptake into astrocytes is through polyspecific organic cation transporters (OCTs). Using RT-PCR, we found that astrocytes express OCT1 and 3. Furthermore, we have recently shown that organic cation transporters (OCTs) heterologously expressed in Xenopus oocytes may take up a variety of PAs (Sala-Rabanal et al., 2013). Taken together, we suggest that in resting physiological conditions, when Cx-43 may be kept at low open probability by extracellular calcium and magnesium, the OCTs play a major role for PA uptake. Alternatively, when neuronal firing causes decreases in extracellular concentrations of Ca^{+2} and Na^{+}, the Cx43 pathway for release and uptake of PAs may become important.

Disclosures: Y. Rivera: None. **M. Inyushin:** None. **Y.V. Kucheryavykh:** None. **M. Sala-Rabanal:** None. **L.Y. Kucheryavykh:** None. **J. Benedikt:** None. **A. Zayas-Santiago:** None. **R.W. Veh:** None. **C.G. Nichols:** None. **M.J. Eaton:** None. **S.N. Skatchkov:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.07/G11

Topic: B.11. Glia-Neuron Interactions

Title: Sex differences in glia activity within the periaqueductal gray: Role in pain and analgesia

Authors: *L. N. EIDSON[1], L. M. BUTKOVICH[2], H. H. DOYLE[2], A. Z. MURPHY[2];
[2]Neurosci. Inst., [1]Georgia State Univ., ATLANTA, GA

Abstract: While morphine is one of the most effective analgesics available, females typically require 2-3 times more morphine than males to experience the same level of analgesia. The midbrain periaqueductal gray (PAG) is a central locus in pain signaling and opioid action. Recent studies suggest that morphine binds to the innate immune receptor toll-like receptor 4 (TLR4) on glia, resulting in increased activation of microglia and astrocytes and opposition of morphine analgesia. Here we test the hypothesis that the sexually dimorphic effects of morphine are due to sex differences in morphine-induced glial activity in the PAG. Specifically, we hypothesize that morphine increases PAG glial cell activity to a greater extent in females as compared with males, resulting in greater opposition of morphine analgesia.
Male and female rats were sacrificed 15, 30, and 60 minutes following subcutaneous morphine

(or equivolume saline), and tissue from the PAG was stained immunohistochemically to visualize microglial and astrocytic cell activity. Our results support our hypothesis of sex differences in glia expression within the PAG. Specifically, while baseline glia activity in the PAG of male brains exceeded that of females, morphine administration resulted in a significantly greater activation of glia in females in comparison to males at all time points.
To test the hypothesis that sex differences in morphine activation of glia contributed to sex differences in morphine analgesia, a separate group of animals were implanted with bilateral cannulae into the PAG and received infusions of (+)-naloxone (a stereoselective TLR4 antagonist) immediately prior to subcutaneous morphine injections. PAG TLR4 antagonism eliminated sex differences in morphine analgesia, with females responding comparably to male vehicle controls. These results suggest that sex differences in opiate activation of glia contribute to the sexually dimorphic actions of morphine, and that inhibition of PAG glia activation will facilitate morphine analgesia in females.

Disclosures: L.N. Eidson: None. **L.M. Butkovich:** None. **H.H. Doyle:** None. **A.Z. Murphy:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.08/G12

Topic: B.11. Glia-Neuron Interactions

Support: NIH Grant NS46606

NCI Grant CA124787

Title: Astrocytes are activated in the absence of microglial activation in chemotherapy-induced peripheral neuropathy models in rats

Authors: *C. R. ROBINSON, P. M. DOUGHERTY;
Pain Med., UT MD Anderson Cancer Ctr., Houston, TX

Abstract: Activation of glial cells has been shown to correlate with the development and ongoing presence of multiple chronic pain models, particularly in the context of nerve injury. While microglia are active in these injury models, they are not active in chemotherapy-induced pain models. However, it was recently shown that astrocytes are active in paclitaxel-induced peripheral neuropathy in rats. The aim of the present study was to determine whether this trend of astrocytic activation holds true in other chemotherapy models of pain. Spinal cords from male

Sprague-Dawley rats were used for all experiments. Astrocytic and microglial activation was quantified across time points corresponding to the induction, maintenance, and extinction of hyper-reflexive behavioral phenotypes in oxaliplatin, bortezomib, and chronic constriction injury (CCI) models. Additionally, glial activity was quantified at lumbar, thoracic, cervical, and midbrain levels in these models to determine whether glial activation was localized to regions corresponding to hyper-reflexive dermatomes. It was found that astrocytes were activated only at early time points following oxaliplatin treatment, but showed persistent activation following bortezomib treatment in a time course parallel to changes in behavior. Microglia were not activated at any time point in any of the chemotherapy models, but did show activation in the CCI model. Additional experiments to be presented in the poster will include a more subtle assay into the activation state of microglia in these models. In addition to staining with OX-42, additional staining will include markers to M1 and M2 activation states of microglia to determine whether there is a more subtle change in the functional state of microglia.

Disclosures: C.R. Robinson: None. **P.M. Dougherty:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.09/G13

Topic: B.11. Glia-Neuron Interactions

Support: GACR grant P303/11/2378

GACR grant 13-02154S

GACR grant P304/12/G069

Title: Changes in astroglial volume, extracellular space geometry and K+ concentration in α-syntrophin deficient mice during cellular swelling

Authors: *L. VARGOVA[1,2], M. CICANIC[1,2], L. DMYTRENKO[1,2], J. TURECKOVA[3], M. ANDEROVA[3,1];
[1]Dept. of Neurosci., Charles University, 2nd Med. Fac., Prague, Czech Republic; [2]Dept. of Neurosci., [3]Dept. of Cell. Neurophysiol., Inst. of Exptl. Med., Prague, Czech Republic

Abstract: Water transport via the aquaporin-4 channel (AQP4) located in the perivascular and subpial astroglial processes contributes to physiological and pathological volume changes of the extracellular space (ECS) affecting ECS ionic and osmotic balance and thus neuronal

excitability. AQP4 is anchored by α-syntrophin (α-syn), thus making α-syn deficient mice (α-syn -/-) a useful model for studying the role of AQP4 in astroglial volume homeostasis. In order to visualize and quantify morphological changes in astrocytes that lack α-syn, double transgenic mice (DT) were generated by crossbreeding GFAP/EGFP mice with α-syn -/- mice. GFAP/EGFP mice were used as a control group ("wild type" - wt). Measurements were performed in the somatosensory cortex of DT and age-matched wt mice. Astroglial volume changes were assessed by 3D-confocal morphometry, and the ECS volume fraction α (α = ECS volume/total tissue volume) and tortuosity λ (λ2 = apparent diffusion coefficient/free diffusion coefficient) were measured using the real-time iontophoretic method. Experimental models of physiological and pathological cell swelling included: 30 min superfusion of brain slices by hypotonic solution (250 and 200 mOsm) and solutions with elevated K+ (10 and 50 mM).
Control values of α were significantly smaller in wt mice (0.19 ± 0.01, N = 16, N - number of animals) than in DT animals (0.22 ± 0.01, N = 19) with no significant difference in λ. While the relative decrease after superfusion with 250 mOsm solution did not differ between wt and DT mice, the ECS shrinkage induced by 200 mOsm solution as well as by a mild increase in K+ (10 mM) was smaller in DT than in wt mice (200 mOsm : by 25 and 42 %, respectively; 10 mM K+: 23 and 35 %, respectively). 50 mM K+ induced a pronounced decrease in α by 70 % in both wt and α-syn -/- mice. Similarly, 3D-confocal morphometry revealed that α-syn deletion results in significantly smaller astrocyte swelling only when induced by more severe stress (200 mOsm or by 50 mM K+). The volume recovery of cortical astrocytes from DT mice was significantly slower following their exposure to 200 mOsm, whereas no differences between wt and DT mice were found in astrocyte volume recovery after perfusion with 50 mM K+. Our results suggest that AQP4 channels contribute to astroglial swelling and changes in the ECS volume, especially during more severe insults. These alterations may affect the concentration and diffusion of neuroactive substances and thus influence the level of tissue damage during brain edema.

Disclosures: L. Vargova: None. **M. Cicanic:** None. **L. Dmytrenko:** None. **J. Tureckova:** None. **M. Anderova:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.10/G14

Topic: B.11. Glia-Neuron Interactions

Support: WSU Incentive to Collaborate Grant

Title: Stimulation-induced volume changes of the CA1 region in rat hippocampal slices

Authors: A. B. GUTWEIN[1], *J. E. OLSON[2];
[1]Dept Neuroscience, Cell Biol. and Physiol., [2]Dept Emergency Med., Wright State Univ. Boonshoft Sch. Med., Dayton, OH

Abstract: Changes in brain extracellular and intracellular volumes occur during pathophysiological conditions; however, mechanisms of cell swelling and volume regulation during functional brain activity are unclear. To examine these processes, volume changes in stratum radiatum of the CA1 region of 400 µm Sprague-Dawley rat hippocampal slices were evaluated during stimulation of Schaffer collaterals. Field potentials in the CA1 pyramidal layer were monitored to assure slice viability and evaluate drug actions. The stimulation strength of 200 µsec bipolar pulses was adjusted to the lowest current which produced the maximal field potential (0.2-0.9 mA). We characterized changes hippocampal volume by irradiating slices with white light and acquiring images every 10 sec to measure light transmission through the tissue (intrinsic optical signal or IOS). Slices were perfused with artificial cerebrospinal fluid (aCSF) and the effects of synaptic activity, ionotropic glutamate receptor (iGluR) activation, glutamate uptake, and volume regulated anion channels (VRAC) were examined by altering the aCSF composition or by adding drugs. Data were analyzed using ANOVA with Dunnett's post hoc test ($p<0.05$ indicated significance). We found IOS increased with stimulation frequency between 1 Hz and 10 Hz. Stimulation for 5 min at 10 Hz was selected for the remaining experiments because it produced consistent and significant swelling. With this stimulation frequency, IOS initially increased from baseline by 2.21 ± 0.72% per min. IOS returned to the baseline value 5 min after the stimulation train with an initial rate of recovery of -1.26 ± 0.32% per min. When synaptic activity was blocked by perfusing the slice with high Mg+2/low Ca+2 aCSF, the initial IOS rate of change with stimulation was reduced by 81% and IOS did fully recover following the stimulation train. CNQX and MK-801 (iGluR AMPA and NMDA antagonists, respectively) did not affect the initial IOS rate of change with stimulation, but did inhibit the initial IOS rate of recovery after the stimulation train by 52% and 59%, respectively. Inhibition of EAAT1 and EAAT2 mediated glutamate uptake with TFB-TBOA reduced the initial IOS rate of change with stimulation by 70%, similar to that observed with synaptic block. VRAC inhibition with niflumic acid, NPPB, and DCPIB caused various stimulation-induced changes in IOS suggesting the responses were caused by effects on components other than VRAC. We conclude the majority of swelling during stimulation is due to glutamate accumulation. Ionotropic glutamate receptors play a role in initiating volume recovery following stimulation; however, recovery is not dependent on VRAC activity.

Disclosures: A.B. Gutwein: None. **J.E. Olson:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.11/G15

Topic: B.11. Glia-Neuron Interactions

Title: The role of astrocytic swelling on neuronal excitability: Implications for both cerebral edema and epilepsy

Authors: *K. LAUDERDALE, T. FIACCO;
Univ. of California Riverside, Riverside, CA

Abstract: Cerebral edema affects millions of people worldwide suffering from various diseases, disorders, and injuries such as stroke, cardiac arrest, epilepsy, autism, and traumatic brain injury. Edema is characterized by swelling of the brain tissue, increased intracranial pressure, reduced cerebral blood flow and can result in seizures, cerebral herniation, and death. It has been known for some time that cell swelling and reduction of the extracellular space can lead to increases in neuronal excitability and seizures in vitro and in vivo. The elevated neuronal excitability has mainly been attributed to increased ephaptic interactions between neurons. However, astrocytes may contribute actively to this process due to their selective expression of the glial water channel aquaporin 4, together with evidence that astrocyte swelling in vitro leads to significant amounts of glutamate release through astrocytic volume-regulated anion channels. Here we provide evidence suggesting that increased neuronal excitability is an active process triggered by astrocytic swelling specifically. Astrocyte swelling was evoked using a hypoosmolar ACSF solution, during simultaneous recording of activity in CA1 pyramidal neurons in acute mouse hippocampal slices in vitro. Astrocyte swelling produced the following excitatory effects in neurons: large amplitude slow inward currents (SICs), mixed excitatory postsynaptic currents (sEPSCs)/miniature EPSCs (mEPSCs), low amplitude slow inward currents (LA SICs), outward currents (OCs), and seizure-like discharges. Neuronal activity was increased during astrocytic swelling in a dose dependent manner with greater reductions in osmolarity resulting in increased neuronal excitability. Astrocytic swelling was capable of inducing neuronal firing of action potentials independent of AMPA receptor activation. Neuronal excitability was also increased during astrocytic swelling independent of neuronal action potentials and AMPA receptor activation. Taken together, these results indicate that selective astrocyte swelling in hypoosmolar conditions actively contributes to neuronal excitability by activating several different types of receptors. These findings may have important implications for the treatment of numerous conditions associated with cellular swelling and excitotoxicity including stroke, ischemia, traumatic brain injury, hyponatremia, inflammatory diseases, and epilepsy. Additional experiments are underway to determine if this astrocytic pathway contributes forms of epileptiform activity including interictal and ictal discharges, status epilepticus and spontaneously recurring seizures.

Disclosures: K. Lauderdale: None. **T. Fiacco:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.12/G16

Topic: B.11. Glia-Neuron Interactions

Title: Seizures and swelling: What role does astrocyte volume change play in high [K+]o-induced epileptiform activity?

Authors: *T. R. MURPHY, T. A. FIACCO;
Cell Biol. and Neurosci., Univ. of California, Riverside, Riverside, CA

Abstract: Epilepsy, a neurological disorder characterized by recurrent seizures, affects nearly 3 million Americans alone and nearly 500 new cases are diagnosed each day. Of these cases, two-thirds have an unknown cause, and over 40% cannot be controlled with current therapies (http://www.cureepilepsy.org). Neurologically, these seizures are the result of global hyperexcitability in CNS neurons. However, the cellular and molecular causes of this hyperexcitability are multifactorial and not well understood. Astrocytes have the ability to respond to synaptic neurotransmitters (Porter and McCarthy 1996) and release neurotransmitters under certain conditions. One such condition is through direct cell swelling during which astrocytes can regulate their volume through the opening of volume-sensitive anion channels (VRACs) that are permeable to amino acid transmitters including glutamate (Rutledge et al 1998). We hypothesize that astrocyte swelling and subsequent glutamate release contribute to the initiation and/or maintenance of the hypersynchronous discharges that underlie seizures. Utilizing a high $[K+]_o$ model of seizure-like activity in vitro, we have established a $[K+]_o$ threshold capable of initiating large, repetitive NMDA-dependent currents in hippocampal CA1 neurons. These currents appear to be the voltage-clamp correlate of paroxysmal depolarization shifts (PDSs) recorded extracellularly in other studies (forming the "interictal" part of a seizure). These discharges occur in the presence of the AMPA receptor antagonist NBQX but are blocked entirely by the NMDAR antagonist AP5. The PDSs appear sensitive to astrocytic VRAC activation, as the astrocyte-specific VRAC inhibitor DCPIB reduces them over time. To further investigate the role of astrocytic swelling-activated glutamate release, we plan to selectively block astrocyte swelling via "volume-clamp" using a patch pipette, and also to directly image astrocyte swelling in high $[K+]_o$ via confocal imaging. Directly loading astrocytes with high levels of glutamate, and recording the result on epileptiform currents will also provide a direct connection between astrocytes and PDSs. Future work will examine changes in threshold or severity of epileptiform activity induced in control versus seizure-prone mice. Additionally, we

plan on developing a model of the “ictal” portion of a seizure, a crucial part of understanding the astrocytes’ role in the development of epilepsy.

Disclosures: T.R. Murphy: None. **T.A. Fiacco:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.13/G17

Topic: B.11. Glia-Neuron Interactions

Title: Glial aquaporin-4 water channels in substantia nigra: Implications for parkinson's disease

Authors: K. STAHL[1], A. PRYDZ[1], M. NADEEM[1], N. DAVARPANEH[1], *M. AMIRY-MOGHADDAM[2];
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Abstract: Aquaporin-4 (AQP4) is the predominant water channel in the brain. Here, it is selectively expressed in astrocyte endfeet facing microvessels, in a highly polarized manner. The polarized expression of AQP4 in astrocytes is crucial for homeostatic processes maintaining normal neuronal function, including water and potassium homeostasis. We have previously shown that loss of AQP4 polarity is associated with several neurological conditions, including stroke, epilepsy and neurodegenerative diseases, such as Alzheimer’s disease. The role of AQP4 in Parkinson’s disease (PD), however, remains largely unexplored. In this study, we aim to investigate the distribution and function of AQP4 in normal substantia nigra (SN) and in animal models of PD.
The distribution of AQP4 in the SN is compared to the cerebral cortex in normal C57BL/6 mice, and in a mouse model of PD, where 1-methyl-4-phenylpyridinium (MPP+) is injected intrastriatally and kept for 7 days in vivo. All animals are perfusion fixed and brains are analyzed using and confocal immunofluorescence and quantitative immunogold electron microscopy. When characterizing the expression of AQP4 under normal conditions, we find significantly higher levels of AQP4 in the SN, as compared to the cerebral cortex. The abundant AQP4 expression is highly polarized around perivascular processes, and there is also substantially more AQP4 in astrocyte processes throughout the neuropil, compared to the cerebral cortex. The high density and polarized expression of AQP4 around perivascular endfeet and processes in the neuropil suggest that AQP4 might play an important role for maintenance of neuronal function in

the SN. We are currently investigating expression patterns of AQP4 in animal models of PD to study its potential role in the PD pathophysiology.

Disclosures: K. Stahl: None. **A. Prydz:** None. **M. Nadeem:** None. **N. Davarpaneh:** None. **M. Amiry-Moghaddam:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.14/G18

Topic: B.11. Glia-Neuron Interactions

Support: JSPS KAKENHI Grant Number 23790994

Title: NMO sera down-regulate the expression of aqp4 in human astrocyte and induce cytotoxicity independent of complement

Authors: *H. HARUKI[1,2], Y. SANO[2], F. SHIMIZU[2], M. OMOTO[2], A. TASAKI[2], M. OISHI[2], M. KOGA[2], T. FUKUSAKO[1], T. KANDA[2];
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Abstract: Background: Autoantibodies against astrocyte water channel aquaporin-4 (AQP4) are highly specific for neuromyelitis optica (NMO). However, to date, the molecular mechanism of NMO is unclear. Although AQP4 exists as two different heterotetramers, M-1 and M-23 AQP4, which result from usage of different start codons and vary in the 23 amino acids in the N terminus of the protein, *in vitro* study using human astrocyte, particularly the cells expressing dominantly M23 isoform, is rare. The purpose of this study was to identify the possible humoral mechanisms responsible for the astrocytic damage.
Methods: Human primary astrocyte (AST) was immortalized by retroviral vectors harboring temperature-sensitive SV40 T antigen gene and AQP4 cDNA (M23), designated as hAST-AQP4. This line was used to determine whether NMO sera influence the amount of AQP4, excitatory amino acid transporter 2 (EAAT2) as construct AQP4-associated protein-complexes and inflammatory cytokines. In addition, the effects of NMO sera on the morphology and viability of astrocytes and the subcellular localization of AQP4 were investigated.
Results: After exposure of NMO sera, hAST-AQP4 exhibited extension of narrow processes and surface membrane-bound AQP4 proteins were gathered and appeared punctate, dot-like structures. NMO sera alone induced cytotoxicity and addition of complement had more harmful effect on hAST-AQP4. NMO sera decreased AQP4 mRNA and protein. The amount of EAAT2

in the human astrocytes was significantly decreased following exposure to NMO sera. NMO sera alone up-regulated TNFα and IL-6 in astrocytes and co-incubation with anti-TNFα and anti-IL-6 neutralizing antibodies blocked both the cytotoxicity and reduction of AQP4 in astrocytes. **Conclusions:** NMO sera solely could bring cytotoxicity on human astrocyte, involving AQP4 reduction and inflammatory cytokine up-regulation. The future elucidation of factors that block the production of inflammatory cytokines may therefore lead to the development of a novel therapeutic strategy.

Disclosures: H. Haruki: None. **Y. Sano:** None. **F. Shimizu:** None. **M. Omoto:** None. **A. Tasaki:** None. **M. Oishi:** None. **M. Koga:** None. **T. Fukusako:** None. **T. Kanda:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.15/G19

Topic: B.11. Glia-Neuron Interactions

Support: Healty Minister LR 2012/2013

Title: Targeting inflammasome activation is a promising strategy to control seizures induction and recurrence

Authors: *L. LIBRIZZI, F. M. NOÈ, M. DE CURTIS;
Dept of Exptl. Neurofisiology and Epilettology, Fondazione Inst. Neurologico C. Besta, Milan, Italy

Abstract: Associated brain inflammatory response occur in human focal epilepsies and promote the generation of seizures in experimental models. Pro-inflammatory mediator interleukin (IL)-1beta contribute to seizure generation, and pharmacological blockade of its cognate receptor IL-1R1 reduces experimental seizures [Vezzani, Brain Behaviour & Immunity 25:1281,2011; Vezzani, Nat Rev Neurol 7:31,2011; Librizzi, Ann Neurol xx 2012]. IL-1b biosynthesis and release is pivotally controlled by the assembly of a macromolecular complex called NLRP3 inflammasome, consisting of regulatory, adaptor and effector subunits (Schroder & Tschopp, Cell 140:821,2010). ATP released during seizures, activates the NLRP3 inflammasome by stimulating the ionotropic P2X7 purinergic receptor [Mariathasan, Nature 9:228,2006]. ATP-mediated P2X7 receptor activation results opening of Pannexin-1 pore, thus leading to K efflux and intracellular Ca rise. In the present study we investigated if seizure-induced brain born inflammation and BBB damage are counteracted by a cocktail of P2X7 receptor/ Pannexin-1 complex inhibitors.

Epileptiform activity was induced by arterial perfusion with either the GABAA receptor antagonist bicuculline, or the potassium channel blocker 4-aminopyridine (4AP).
Brief application of bicuculline (50µM) consistently induced focal ictal discharge in the limbic region, as verified by simultaneous electrophysiological recordings of extracellular activity in medial entorhinal cortex (mERC) and CA1.
Arterial application of 4AP (50 µM) induced independent seizure like activities in both the olfactory region and the limbic region characterized by fast activity at onset followed by irregular spiking and rhythmic bursting (Carriero et al., 2010). In the piryform cortex (PC), we observed runs of fast activity (fast runs, FRs) that progressively became more robust and ultimately generated all-or-none seizure-like events (SLE).
The 30-min infusion via the resident arterial system of the P2X7 receptor/ Pannexin-1 complex inhibitors (A438079 100µM and Probenecid 1mM, respectively) initiated from 5 min before bicuculline or 4AP application, drastically counteract epileptiform discharges in mERC and totally abolished the FRs and SLE described in the PC.
This set of evidence suggests that NLRP3 inflammasome system may be critically involved in aberrant neuronal excitability and seizures, and its selective antagonism, through the upstream inhibition of the P2X7receptors/pannexin 1 complex, may be a promising strategy to control seizures in focal epilepsies.

Disclosures: L. Librizzi: None. **F.M. Noè:** None. **M. de Curtis:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.16/G20

Topic: B.11. Glia-Neuron Interactions

Title: Complement inhibitor protein cd59 modulates cytotoxicity and inflammation in neuromyelitis optica

Authors: *H. ZHANG, A. S. VERKMAN;
Dept of Med., UCSF, SAN FRANCISCO, CA

Abstract: Neuromyelitis optica (NMO) is an autoimmune disorder or the central nervous system in which inflammatory demyelinating lesions damage spinal cord and optic nerve, and, to a lesser extent, brain. NMO pathogenesis involves binding of immunoglobulin G anti-aquaporin-4 autoantibodies (NMO-IgG) to astrocytes, causing complement-dependent cytotoxicity (CDC) and secondary inflammation, demyelination and neuron loss. Based on the central role of

complement in NMO, we investigated the involvement of the major astrocyte complement inhibitor CD59, a phosphoinositol-linked glycoprotein that inhibits the terminal C5b-9 membrane attack complex. Immunofluorescence showed strong CD59 expression at sites of aquaporin-4 expression in spinal cord, optic nerve and brain, as well as in peripheral organs including kidney. CD59 inhibition by a neutralizing monoclonal antibody greatly increased NMO-IgG-dependent CDC in primary murine astrocyte cultures, and greatly increased NMO pathology in ex vivo spinal cord slice cultures exposed to NMO-IgG and complement, causing marked demyelination. Greatly increased NMO pathology was also found in spinal cord slice cultures from CD59 knockout mice and in vivo following intracerebral injection of NMO-IgG and human complement. We found that intrathecal injection (at L5-L6) of small amounts NMO-IgG and complement in CD59-deficient mice produced robust, longitudinally extensive white matter lesions throughout the thoracic and lumbar spinal cord segments of spinal cord, whereas minimal pathology was seen in wild type mice. The lesions were most severe at 2 days after injection, showing loss of AQP4 and GFAP, demyelination, activation of microglia, infiltration of leukocytes, and remarkable loss of pain nociception in hindpaws. Our results implicate CD59 as an important modulator of the immune response in NMO, and provide a novel animal model of NMO that closely recapitulates human NMO pathology. Upregulation of CD59 on astrocytes may have therapeutic benefit in NMO.

Disclosures: H. Zhang: None. **A.S. Verkman:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.17/G21

Topic: B.11. Glia-Neuron Interactions

Support: NSC 101-2321-B-010 -022 from National Science Council, Taiwan

Aim for the Top University Plan, Ministry of Education, Taiwan

Title: Roles of aryl hydrocarbon receptor in the lipopolysaccharide-induced astrogliosis

Authors: Y.-L. GAN[1], P.-Y. LEE[1], P.-C. HSU[2], Y.-P. YEH[1], *Y.-H. LEE[1,2];
[1]Inst. of Physiol., [2]Brain Res. Ctr., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Inflammatory insult-induced astrocyte reactivation plays important roles in the inflammation and injury repair after brain injuries. Previous studies have shown that kynurenine (Kyn) and kynurenic acid (KyA) are detected in the blood of post-stroke patients, which points to

the possible activation of their synthesizing enzyme indoleamine 2,3-dioxygenase (IDO). IDO catalyzes the biosynthesis of Kyn from L-tryptophan, and this metabolic pathway has immune modulatory effect on peripheral immune responses. IDO is inducible by proinflammatory insults such as bacterial endotoxin lipopolysaccharide (LPS), and has been implicated in the pathogenesis of neurodegeneration. Recent studies have identified that Kyn and KyA are the endogenous ligand of the aryl hydrocarbon receptor (AhR), also known as the receptor for the environmental hormone dioxin. AhR plays important roles in immune regulation, but whether it is involved in the IDO-mediated immune modulation in CNS remains unknown. In this study, we investigated the role of AhR in the IDO/Kyn pathway and proinflammatory cytokine expression in LPS-induced astrocyte reactivation. Immunofluorescent double labeling indicated that intracerebroventricular administration of LPS induced the expression of AhR and IDO in rat brains. Western blotting and RT-PCR results also showed that both AhR and IDO were induced by LPS in cultured astrocytes, and AhR knockdown profoundly enhanced the LPS-induced IDO expression. *In vivo* study further indicated that LPS-induced IDO expression was higher in AhR-deficient mice compared with the heterozygous and wild type mice. Furthermore, LPS treatment can activate AhR accompanied by the elevation of endogenous Kyn production, and exogenous Kyn application can activate AhR. As a result, the LPS-induced proinflammatory cytokine IL-6/TNFalpha expression was found negatively regulated by the LPS-induced AhR and IDO expression. In contrast, LPS-induced astrocyte hypertrophy was attenuated by AhR knockdown. Together, our data suggest that LPS-induced astrogliosis involves the activation of IDO to synthesize AhR ligand Kyn, which in turn activates AhR to negatively feedback the LPS-induced IDO and proinflammatory expression in order to control the astrocyte-mediated neuroinflammation.

Disclosures: Y. Gan: None. **P. Lee:** None. **Y. Yeh:** None. **Y. Lee:** None. **P. Hsu:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.18/G22

Topic: B.11. Glia-Neuron Interactions

Support: the Mid-career Researcher Program through the National Research Foundation of Korea (NRF) grant funded by the MEST (2011-0028319)

Title: Induction of Krüppel-like factor 4 expression in reactive astrocytes following ischemic injury *In vitro* and *In vivo*

Authors: PARK, T.-R. RIEW, Y.-J. SHIN, J.-M. PARK, J. CHO, M.-Y. LEE;
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Abstract: Krüppel-like factor 4 (KLF4) is a transcription factor with diverse and cell-type specific functions and is linked to a variety of pathophysiological processes. Recently, it has been proposed that regulation of KLF4 is critical to neuronal differentiation and that neural progenitors with KLF4 overexpression form cells with a glial identity. The present study was designed to determine whether KLF4 is involved in astroglial reaction induced by ischemia-reperfusion injury in brain. Astroglial reaction characterized by glial fibrillary acidic protein upregulation and astrogliosis participates in the pathogenesis of neurological disorders. No specific KLF4 immunoreactivity was expressed in resting astrocytes of the control hippocampus, but significant induction was detected in reactive astrocytes preferentially located in the CA1 and dentate hilar regions of the hippocampus following transient forebrain ischemia. Astrogial KLF4 expression was induced in their nuclei and cytoplasm within three days after ischemia, and maintained for at least four weeks. This pattern is reproduced in *in vitro* astrogliosis model of rat primary cortical astrocytes exposed to oxygen-glucose deprivation (OGD). Further, immunoblot assay showed that nuclear and cytosolic extracts from cortical astrocytes subjected to OGD displayed a significant increase of KLF4 protein compared with normoxic extracts. Thus, our data demonstrated that KLF4 expression was induced in astroglia becoming reactive elicited by ischemic injury in vivo and in vitro, suggesting that KLF4 may act as a transcription factor linked to the regulation of the astroglial reaction after ischemic injury.
This study was supported by the Mid-career Researcher Program through the National Research Foundation of Korea (NRF) grant funded by the MEST (2011-0028319).
The first two authors contributed equally to this study.

Disclosures: Park: None. **T. Riew:** None. **Y. Shin:** None. **J. Park:** None. **J. Cho:** None. **M. Lee:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.19/G23

Topic: B.11. Glia-Neuron Interactions

Support: KAKENHI (23115521)

KAKENHI (22500362)

Title: Optogenetic glial alkalization relieves ischemic brain damage

Authors: *K. BEPPU[1], T. SASAKI[1], K. F. TANAKA[2], A. YAMANAKA[3], Y. FUKAZAWA[4], R. SHIGEMOTO[1], K. MATSUI[1,5];
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Abstract: The brain demands high-energy supply and obstruction of blood flow causes rapid deterioration of the healthiness of brain cells. Two major events occur upon ischemia; acidosis and glutamate excitotoxicity. Aerobic metabolism shuts down as a consequence of oxygen and glucose deprivation; however, lactate production from glycogen stored predominantly in glial cells continues, which leads to severe acidosis. Neuronal cell death via glutamate excitotoxicity follows; however, cellular source of glutamate and mechanism of its release remain uncertain. Here we show a causal relationship between glial acidosis and neuronal excitotoxicity. As the major cation that flows through channelrhodopsin-2 (ChR2) is proton, this could be regarded as an optogenetic tool for instant intracellular acidification. Optical activation of ChR2 expressed in cerebellar glial cells led to glial acidification and to activation of glutamate receptors on Purkinje cells in acute brain slices. Depolarization alone by local application of potassium was insufficient to cause glial glutamate release and intracellular calcium chelation did not stop glutamate release caused by the glial ChR2 activation. On the other hand, glial alkalization via optogenetic activation of a proton pump, archaerhodopsin (ArchT), led to cessation of glutamate response in Purkinje cells during oxygen/glucose deprivation in acute slices and to relief of ischemic brain damage in vivo. Our results suggest that glial acidification is a key trigger for glutamate release from glia upon ischemia and, thus, devising a way to control glial pH may be an effective therapeutic strategy for relieving ischemic neuronal cell death.

Disclosures: K. Beppu: None. **T. Sasaki:** None. **K.F. Tanaka:** None. **A. Yamanaka:** None. **Y. Fukazawa:** None. **R. Shigemoto:** None. **K. Matsui:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.20/G24

Topic: B.11. Glia-Neuron Interactions

Support: NIH Grant AG027297

KSCHIRT Grants

Gift from Jeff and Patti Tautenhan

Title: Inhibition of astrocytic calcineurin/NFAT activity protects hippocampal synaptic function in an intact rat model of traumatic brain injury

Authors: *C. M. NORRIS[1], J. L. FURMAN[2], M. M. PLEISS[3], T. L. SUDDUTH[2], D. M. WILCOCK[2], S. W. SCHEFF[2];
[1]Sanders-Brown Ctr. Aging, Univ. Kentucky, LEXINGTON, KY; [2]Sanders-Brown Ctr. on Aging, [3]Mol. and Biomed. Pharmacol., Univ. of Kentucky Col. of Med., Lexington, KY

Abstract:
Astrocyte activation is associated with acute injury and found in nearly every chronic neurodegenerative disease. Nevertheless, the impact of activated astrocytes on neurologic function remains poorly understood. The activated astrocyte phenotype appears to be regulated, in part, by the protein phosphatase calcineurin (CN) and the CN-dependent transcription factor, NFAT. Consistent with previous work on primary neural culture models, we recently showed that selective inhibition of astrocytic CN/NFAT signaling using adeno-associated virus (AAV) vectors suppressed morphologic features of astrocyte activation in an intact mouse model of Alzheimer's disease (AD). Importantly, these changes were associated with reduced microglial activation, reduced Abeta pathology, and improved cognitive and synaptic function. The results suggest that activated astrocytes, in general, and astrocytic CN/NFAT activity, in particular, negatively regulate neurologic function under some degenerative conditions. In the present study, we determined the extent to which astrocytic CN/NFAT activity contributes to functional outcome in a rat model of cortical contusion injury (CCI). Adult rats received bi-lateral hippocampal injections of AAV vectors expressing the human GFAP promoter (Gfa2) and the potent CN/NFAT inhibitor, VIVIT. At 2-3 months post-infection, rats received a unilateral CCI and were permitted to recover for one week. Hippocampal slices were then prepared from injured and uninjured hemispheres for the electrophysiologic assessment of CA3-CA1 synaptic strength and susceptibility to long-term depression (LTD) obtained under investigator-blind conditions. Relative to slices from the uninjured hemisphere and slices from sham animals, slices from the injured hemisphere of control rats (i.e. rats treated with a control AAV construct--AAV-Gfa2-EGFP) exhibited significantly reduced basal synaptic strength and robust LTD. In contrast, synaptic strength in rats treated with AAV-Gfa2-VIVIT was very comparable across the injured and uninjured hemispheres and nearly indistinguishable from sham rat slices. Moreover, slices from AAV-Gfa2-VIVIT rats showed almost no LTD. Finally, AAV-Gfa2-VIVIT provided significant protection from the loss of GluR1 receptors characteristic of injured hippocampal tissue of control rats. Thus, similar to results with AD mouse models, activated astrocytes appear to contribute to synaptic dysfunction in a model of acute traumatic brain injury. Ongoing studies

are determining the extent to which astrocytic CN/NFAT activity regulates neuroinflammatory markers following CCI.

Disclosures: C.M. Norris: None. **J.L. Furman:** None. **M.M. Pleiss:** None. **T.L. Sudduth:** None. **D.M. Wilcock:** None. **S.W. Scheff:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.21/G25

Topic: B.11. Glia-Neuron Interactions

Support: NIH grant RO1MH067121

Title: Synaptogenic effects of astrocytic ephrinB1 in the adult mouse hippocampus following severe traumatic brain injury

Authors: *A. M. NIKOLAKOPOULOU[1], J. LEISH[1], S. MORTAZAVI[1], A. OBENAUS[2], I. M. ETHELL[1];
[1]Biomed. Sci., Univ. of California Riverside, Riverside, CA; [2]Pediatrics, Loma Linda Univ., Loma Linda, CA

Abstract: Molecular and cellular mechanisms of neuron-glial interactions that regulate brain repair following traumatic brain injury (TBI) have attracted the attention of many investigators. EphrinB1 was identified as one of the genes that are upregulated in reactive astrocytes and may promote or hinder regeneration of brain circuits after brain injury. Here, we focus our studies on the involvement of ephrinB1/EphB2 receptor signaling in reactive synaptogenesis and brain repair following TBI. We tested ephrinB1 expression in mouse hippocampi at 1, 3 and 7 days post TBI using a controlled cortical impact model and observed a significant increase in ephrinB1 protein levels in hippocampal astrocytes at 3 days post-TBI. Astrocytic upregulation of ephrinB1 was mostly noted in the stratum radiatum (SR), whilst the hilus of the dentate gyrus showed a moderate increase in the number of GFAP positive astrocytes and ephrinB1-expressing cells. Upregulation of ephrinB1 levels at 3 days post TBI was followed by a significant downregulation of astrocytic ephrinB1 at 7 days post TBI that coincided with a peak of reactive astrogliosis. We have also found a significant downregulation in the number of glutamatergic synapses in SR at 3 days post TBI in both injured and non-injured hemisphere. Interestingly, we

see up-regulation of ephrinB1 in neurons, but not astrocytes, in the cortex suggesting that astrocytic upregulation of ephrinB1 is specific to the hippocampus. We have also examined GFAP, vimentin (markers for reactive astrocytes), ephrinB1 and EphB2 mRNA expression following TBI and our results show an increase in gene expression following TBI. We are currently investigating the effects of specific ephrinB1 ablation in astrocytes on synaptic rewiring following TBI in the hippocampus of conditional ephrinB1 knockout mice. Our data show that Cre-induced ablation of ephrinB1 in astrocytes increased the number of glutamatergic synapses 3 days post TBI thus implying that ephrinB1 may play a role in trimming excitatory connections following TBI as a protective mechanism against excitotoxicity. Our in vitro studies support this possibility and show that addition of ephrinB1-expressing, but not ephrinB1-deficient, astrocytes to developed neuronal cultures triggers a significant increase in the number of synapses. Our studies suggest that ephrinB1 may play a neuroprotective role in the brain by modulating synaptic connectivity following TBI.

Disclosures: A.M. Nikolakopoulou: None. **J. Leish:** None. **S. Mortazavi:** None. **A. Obenaus:** None. **I.M. Ethell:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.22/G26

Topic: B.11. Glia-Neuron Interactions

Support: National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1B4001262)

Title: Temporal patterns of the embryonic intermediate filament nestin in the hippocampus of mice after TMT treatment

Authors: *S. LEE, J. KIM, J. KIM, Y. SON, S.-H. KIM, J.-C. KIM, T. SHIN, C. MOON; Buk-gu, Chonnam Natl. Univ., Gwangju, Korea, Republic of

Abstract:
Nestin is an embryonic intermediate filament protein that is expressed in multipotent neural stem cells of the central nervous system. In this study, the expression pattern of nestin in the mouse hippocampus 1, 2, 4 and 8 days after trimethyltin (TMT) treatment was examined to elucidate its role in the chemical-induced hippocampal injury. The mRNA level of nestin was significantly increased in the hippocampus 2 days post-treatment, and thereafter, decreased 4 and 8 days post-treatment. The protein level of nestin was significantly increased 4

days post-treatment, and the expression was localized predominantly in GFAP-positive astrocytes in the hippocampal dentate gyrus and CA3. Therefore, we suggest that nestin contributes to the remodeling of the chemically injured region in the hippocampus, possibly through the activated cell migration and the glial scar formation.

Disclosures: S. Lee: None. **J. Kim:** None. **J. Kim:** None. **Y. Son:** None. **S. Kim:** None. **J. Kim:** None. **T. Shin:** None. **C. Moon:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.23/G27

Topic: B.11. Glia-Neuron Interactions

Support: NIH AA017367

Title: Amygdala astrocytic responses to corticotropin-releasing factor and stress

Authors: *L. H. CONTI[1,2], L. M. O'KEEFE[3], S. MACISAAC[3], S. J. CROCKER[3];
[1]Med. Sci., Quinnipiac Univ., Hamden, CT; [2]Psychiatry, [3]Neurosci., Univ. of Connecticut Hlth. Ctr., Farmington, CT

Abstract: Astrocytes are known to play a role in synaptic transmission and along with pre- and post-synaptic neurons form tripartite synapses (Allen and Barres, 2009). The effect of stress on astrocyte function has been studied with mixed results. However, the results of most studies show that astrocytes are activated by stress. The neuropeptide, corticotropin-releasing factor (CRF) is released during stress with behavioral consequences. Both CRF_1 and CRF_2 receptors are found on astrocytes, suggesting the possibility that CRF would have a functional effect on astrocytes in an *in vivo* model. To examine this, Brown Norway rats received either an intracerebroventricular (ICV) infusion of CRF (1.0 μg in 5.0μl) or saline, and brains were harvested 90 min later. To assess astrocyte activation, we examined GFAP immunoreactivity in the basolateral nucleus of the amygdala (BLA). We found that rats which received an ICV infusion of CRF had a significantly greater number of GFAP-positive cells in the BLA than vehicle-treated rats. In a second experiment, rats were either naïve, or underwent 90 min of restraint stress and brains were harvested either immediately, or 24 hr after the termination of restraint. We found a significantly greater number of GFAP-positive cells in the BLA of rats sacrificed 24 hr after stress termination than in either the naïve group or the group that was sacrificed immediately after restraint. Finally, we found that application of CRF to primary astrocyte cultures specifically activated the p38 kinase pathway and enhanced EIF-4E expression

levels. Together, these results implicate astrocytes in the cellular responses to CRF and stress, suggesting that astrocytes may participate in the behavioral responses to CRF and stress.

Disclosures: L.H. Conti: None. **L.M. O'Keefe:** None. **S. MacIsaac:** None. **S.J. Crocker:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.24/G28

Topic: B.11. Glia-Neuron Interactions

Title: Astrocyte activation in pre-pubescent rats stressed by chronic environmental noise

Authors: *O. HUET[1], Y. RUVALCABA-DELGADILLO[1], R. GONZÁLEZ-CASTAÑEDA[1], J. GARCÍA-ESTRADA[2], A. FERIA-VELASCO[1], M. MACÍAS-ISLAS[2], S. LUQUIN[1];
[1]Univ. De Guadalajara, Zapopan, Mexico; [2]IMSS, Guadalajara, Mexico

Abstract: Chronic urban noise can be a deleterious stimulus for those who do not habituate to it, with subsequent adverse health effects. Pre-pubescent individuals who are exposed to this phenomenon go through a critical period in which stressors can alter brain development and long term neurological processes. We previously showed that our noise model works as a moderate stressor which stimulated spatial learning abilities in both, young and adult rats. The aim in the current study was to analyze if astrocytes; cells that regulate neuron function in many ways, and get activated in many central nervous system pathologies, modified their morphology after exposure to chronic environmental noise. Ten recently weaned male Wistar rats were subjected to noise (urban sounds adapted to the rat audiogram) during 15 days, from 21-36 postnatal days. Then, Glial fibrillary acidic protein (GFAP) was analyzed by immunohistochemistry in CA1, CA3 and dentate gyrus regions of hippocampus. The results shown a non-significant increase in the number of GFAP immunopositive cells in all regions, Scholl analysis demonstrated a significant increase in the length of the astrocyte processes of rats exposed to noise. These findings suggest that noise as a moderate stressor can promote learning abilities after astrocyte activation. It may be that astrogliosis has a role in the process of learning under moderate stress.

Disclosures: O. Huet: None. **Y. Ruvalcaba-Delgadillo:** None. **R. González-Castañeda:** None. **J. García-Estrada:** None. **A. Feria-Velasco:** None. **M. Macías-Islas:** None. **S. Luquin:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.25/G29

Topic: B.11. Glia-Neuron Interactions

Support: U.M.S.N.H.-CIC 2013

SEP-ANUIES-CONACYT-ECOS NORD

Title: Expression of the mRNA of the α isoforms of the sodium pump in cerebellum of rats with nutritional stress

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Abstract: The sodium and potassium pump (Na^+/K^+-ATPase) is an enzymatic complex which is located in the plasmatic membrane of all animal , it is constituted by two α subunits, of which there are 4 isoforms (α1, α2, α3 y α4) and 2 β subunits of which are known 3 isoforms (β1, β2 y β3), this enzyme regulates the Na^+ and K^+ ions concentration, it maintains the cellular volume and resets the membrane potential among
others. One the other hand, it is know that serotonin (5-hidroxitryptamine, 5-HT) increase the Na^+/K^+-ATPase activity of glial cells in the cerebellum of rats, and it has been observed an increase in the Vmax of
the enzyme kinetics induced by serotonin and an increase in the ouabain binding to the sodium and potassium pump has been observed in rats' cerebellum with protein-caloric restriction (PCR). Serotonin it is synthesized from the essential amino acid L-tryptophan. Experimentally its synthesis can be activated by administering its precursor or schemes of fetal protein-caloric restrictions. The mechanisms involved in the increase of sodium pump activity by 5-HT is unknown, so, the objective of the present work was to explore using RT-PCR techniques the expression of α isoforms of the enzyme in the cerebellum of
rats with protein-caloric restriction and rats administered with L-Trp. The results showed that in the cerebellum of rats with RPC the expression of mRNA of the a2 subunit was increased in 24.7 % and the enzymatic activity was increased 18.2%. In the cerebellum of rats administered with L-tryptophan the expression of the α2 isoform was increased in 70% and the enzymatic activity was increased in 46.6%. In relationship with the other α isoforms (α1 and α2) they did not show changes in their expression in any group compared with the control group. This data suggest that the 5-HT increase the activity of sodium and potassium pump through the increase of expression

of the α2 catalytic subunit in cerebellum of rats with nutritional stress. This work was partially supported by: U.M.S.N.H.- CIC 26.2 (2013), ANUIES-SEP-CONACyT-ECOS NORD.

Disclosures: R. Mercado: None. **G. Nava:** None. **R. Esquivel:** None. **C.S. Bautista:** None. **O.A. Sifuentes:** None. **O. Guzmán:** None. **F. Bolaños:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.26/G30

Topic: B.11. Glia-Neuron Interactions

Support: CNPq/Brazil Grant 479483/2011-6

FAPESC/PPP #11,338/2012-7

PGFAR

PPGBQA

UFSC

Title: Mechanisms underlying Roundup®-induced neurotoxicity in immature rat hippocampus

Authors: *A. ZAMONER PACHECO DE SOUZA, D. CATTANI, V. L. L. O. CAVALLI, J. T. DOMINGUES, C. E. H. RIEG, C. M. ANDRADE, T. DAL-CIM, C. I. TASCA, F. R. M. B. SILVA;
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Abstract: Glyphosate is the primary active ingredient present in the herbicide Roundup® and is the widely used herbicide worldwide. The present results show that the herbicide Roundup® induces glutamatergic excitotoxicity, oxidative stress and activates multiple signaling pathways leading to neural cell death in rat hippocampus. The pesticide causes Ca^{2+} overload by activating NMDA receptors and L-type voltage-dependent Ca^{2+} channels (L-VDCC) setting off oxidative stress and cell death. The mechanisms underlying Roundup® neurotoxicity involve the activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and extracellular signal-regulated kinase (ERK). Astrocytes play a key role in removing glutamate from the synaptic cleft and metabolizing it to glutamine, which serve as a glutamate precursor in neurons. Our results demonstrated that Roundup® reduced glutamate uptake and metabolism within glial cells, associated with increased release of this neurotransmitter in the synaptic cleft. Moreover,

Roundup® leads to glutamine synthetase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymatic activity inhibition, attesting that either glutamate uptake or its metabolism is impaired in hippocampal astrocytes exposed to the herbicide. Moreover, Roundup® reduced GSH levels and increased the amounts of thiobarbituric acid reactive species (TBARS), characterizing oxidative damage. Also, exposure to the pesticide decreased the activity of gamma-glutamyl transferase (GGT) and glucose-6-phosphate dehydrogenase, supporting the depletion of GSH. In this context, the GGT inhibition induced by Roundup® could decrease the amino acid availability to GSH *de novo* synthesis and the decreased activity of G6PD may reduce NADPH levels, necessary to reduce glutathione. Taken together these results demonstrated that Roundup® might lead to excessive extracellular glutamate levels and consequently to excitotoxic condition and energetic deficit in rat hippocampus.

Disclosures: A. Zamoner Pacheco De Souza: None. **D. Cattani:** None. **V.L.L.O. Cavalli:** None. **J.T. Domingues:** None. **C.E.H. Rieg:** None. **C.M. Andrade:** None. **T. Dal-Cim:** None. **C.I. Tasca:** None. **F.R.M.B. Silva:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.27/G31

Topic: B.11. Glia-Neuron Interactions

Support: Heart & Stroke Foundation of Canada

NIH GM-55632

Title: Connexin43 phosphorylation impacts neuroprotection

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Abstract: One of the major ways astrocytes communicate is via gap junctions, unique membrane channels that allow for intercellular and extracellular signaling. Gap junctions are composed primarily of connexin43 (Cx43), a gap junction protein which has been shown to be both beneficial and detrimental in different models of neurotoxicity. With regard to models of ischemia, several reports have demonstrated that Cx43 is an important factor in neuroprotection, however the molecular mechanisms are unclear.

The transmembrane regions of Cx43 are relatively conserved with other connexins, however, the cytoplasmic region is divergent and has been shown to be critical for the regulation of channel and hemichannel activity. The Cx43 C-terminus has been the focus of significant analysis, with putative phosphorylation sites identified using kinases that directly mediate channel gating properties. We have used transgenic mice with mutated phosphorylation sites in a middle cerebral artery stroke model. To examine the role of phosphorylation in neuroprotection, we have used mice with mutations that include the protein kinase C (PKC) site Cx43(S368A), the Casein kinase 1 (CK1) site Cx43(S325A/328Y/330A), and the mitogen activated protein kinase (MAPK) site Cx43(S255/262/279/282A). After 4 days of recovery, brain sections were histologically evaluated for infarct volume. Immunofluorescent analysis of astrocyte reactivity, microglial activation, Cx43 expression, vascular elements and apoptosis were also performed. While we did not observe a significant difference in infarct volume comparing wild type mice to the CK1 transgenic mice, we noted a non-significant increase in PKC mice. More importantly, a significant decrease in infarct volume was measured in MAPK mice. In the penumbra, an increase in astrocyte reactivity was observed in the MAPK mice, compared with wild type mice. Consistent with the infarct volume data, a significant reduction in cell death was also observed in MAPK mice. These results suggests that specifically inhibiting MAPK phosphorylation of Cx43 provides neuroprotection in ischemic conditions, while preventing PKC and CK1 phosphorylation show no protective effect.
Funded by Heart & Stroke Foundation of Canada (CN) and the NIH GM-55632 (PL). MF holds a Heart & Stroke Foundation Fellowship.

Disclosures: C.C. Naus: None. **M. Freitas-Andrade:** None. **J. Bechberger:** None. **B. MacVicar:** None. **P. Lampe:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.28/G32

Topic: B.11. Glia-Neuron Interactions

Support: NIH grant P30EY014801

RPB Career award

Title: Deimination as an astrocytic marker following temperature incubation

Authors: *M. E. ALGECIRAS[1], H. M. SERRA[2], S. K. BHATTACHARYA[1];
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Abstract: Purpose: To determine whether temperature fluctuation elicits an increase in deimination (conversion of protein bound arginine into citrulline) in astrocytes and whether elevated deimination levels can serve as an astrocytic activation marker. Astrocyte activation will be assessed by measuring the expression of a repertoire of established astrocytic markers, including GFAP.
Methods: Isolated brain cortex astrocytes obtained from C57BL/6J mice (about 5000 per plate) were cultured and subjected to different incubation temperatures for one hour, followed by a stabilization period of 23 hours at 37°C. The exposed and control astrocytes were evaluated for deimination levels as well as for levels of other established markers of astrocyte activation using immunohistochemical, Western blot and ELISA analyses.
Results: Optimal growth of astrocytes occurred at 37°C. Hypothermia (31°) treatment showed very few loss of cells (2%) as measured by Tunnel assay. However, 33% cell death was found after hyperthermic incubation (41°) for 1 hour. Decreased GFAP, deimination and peptidylarginine deiminase type 2 (PAD2) levels were found in cells subjected to hypothermia. An increase in some astrocyte activation markers such as Aquaporin4, S100β and Thrombospondin, however, was found in astrocytes subjected to hypothermia. The astrocytes subjected to hyperthermia showed an increase in deimination and PAD2 levels. Furthermore their levels were higher (approximately 3-fold) than for the cells subjected to hypothermia. Hyperthermia was accompanied by an increase in some astrocytic markers such as GFAP, aldehyde dehydrogenase 1 family member L1 (ALDH1L1), J131 and isolevuglandin (ISO[4]LGE2). Mass spectrometric analysis revealed some unique proteins associated with each treatment condition.
Conclusion: The level of deimination undergoes a shift on either side of optimal temperature incubation (37°C) for astrocytic growth. Level of deimination correlates with astrocyte activation markers when cells are subjected to temperature treatment.

Disclosures: M.E. Algeciras: None. **H.M. Serra:** None. **S.K. Bhattacharya:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.29/G33

Topic: B.11. Glia-Neuron Interactions

Support: the Japan Society for the Promotion of Science

Yokoyama Foundation for Clinical Pharmacology

Title: Gαh/TG2 promotes cAMP production accompanied by a modification of adenylylcyclase 8 in human and rat glioma cells

Authors: *Y. OBARA[1,2], Y. YANAGIHATA[2], T. ABE[2], L. DAFIK[3], K. ISHII[1], N. NAKAHATA[2];
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Abstract: Gαh (or transglutaminase-2 (TG2)) is an atypical guanine nucleotide binding-protein that associates with some G protein-coupled receptors including α1-adrenergic receptor, thromboxane A2 receptor, oxytocin receptor and follicle-stimulating hormone receptor. Gαh/TG2 also exerts transglutaminase activity that catalyzes posttranslational protein cross-linking with the formation of ε-(γ-glutamyl) lysine or (γ-glutamyl) polyamine bonds. However, the function of Gαh/TG2 in glial cells remains unclear, we therefore examined the role in signal transduction in detail. In 1321N1 human astrocytoma cells that do not express endogenous Gαh/TG2, overexpression of Gαh/TG2 caused an enhancement of cAMP accumulation stimulated with the β-adrenergic receptor agonist, isoproterenol, or the adenylylcyclase activator, forskolin. This cAMP-enhancement was reversed by the TG2 inhibitor, ERW1069 (Quinolin-3-ylmethyl(S)-1-(((S)-3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(5-fluoro-1H-indol-3-yl)-1-oxopropan-2-ylcarbamate). In rat C6 glioma cells that express endogenous Gαh/TG2, cAMP accumulation induced by isoproterenol or forskolin was significantly inhibited by overexpression of Gαh/TG2-C277V, a dominant-negative mutant that lacks transglutaminase activity, but was not inhibited by the Gαh/TG2-S171E mutant that cannot bind GTP/GDP. These results suggest Gαh/TG2 potentiates adenylylcyclase activity by its transglutaminase activity and not by its G-protein activity. We previously showed that cAMP promoted gene expression of interleukin-6 via cAMP response element in 1321N1 cells (Obara et al., Mol. Pharmacol. 68, 1466-1474, 2005), Gαh/TG2 consistently increased the activities of the cAMP response element and interleukin-6 promoter, accompanied by an enhancement of cAMP in both glioma cells. Since a calmodulin inhibitor, W7, blocked the effect of Gαh/TG2 on cAMP accumulation and calmodulin-sensitive adenylylcyclase 8 mRNA and protein are expressed in both 1321N1 and C6 cells, we focused on post-translational modification of adenylylcyclase 8 by Gαh/TG2. Gαh/TG2 affected neither adenylylcyclase 8 expression levels, glycosylation, nor dimerization status. In contrast, Gαh/TG2 was co-precipitated with adenylylcyclase 8 and pentylamine, a substrate of Gαh/TG2, was incorporated into adenylylcyclase 8 in a transglutaminase activity-dependent manner. Taking these results together, Gαh/TG2 promotes cAMP production accompanied by a modification of adenylylcyclase 8 in glioma cells.

Disclosures: Y. Obara: None. **Y. Yanagihata:** None. **T. Abe:** None. **L. Dafik:** None. **K. Ishii:** None. **N. Nakahata:** None.

Poster

521. **Astrocytes: Injury and Disease**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 521.30/G34

Topic: B.11. Glia-Neuron Interactions

Title: Decreased expression of caveolin-1 in FABP7-deficient astrocytes and its impact on the membrane lipid raft formation

Authors: *Y. KAGAWA, M. EBRAHIMI, K. SHARIFI, A. ISLAM, Y. YASUMOTO, H. MIYAZAKI, S. KAWAMURA, Y. YAMAMOTO, T. SAWADA, N. TOKUDA, Y. OWADA; Dept. of organ anatomy, Yamaguchi Univ. Grad. Sch. of Med., Yamaguchi, Japan

Abstract: [Introduction]Polyunsaturated fatty acids (PUFAs) have an important role as components of cell membrane, and control the cellular lipid metabolism to maintain the cell homeostasis. Fatty acid-binding
proteins 7 (FABP7), which has high affinity with omega-3 PUFAs, is strongly expressed by astrocytes and oligodendrocyte precursor cells in the brain. We previously showed that FABP7-deficiency in mice resulted in altered emotional behavior, and that FABP7 expression was altered in human schizophrenic brains. However, the precise role of FABP7 in astrocyte and how FABP7-deficiency is associated with schizophrenia pathology are still unknown. In this study, we examined the role of FABP7 in the astrocytes focusing on the lipid raft formation, which is essential for various receptor-mediated signal transduction in response to external stimuli. [Methods and Results]In the membrane lipid raft fractions separated by sucrose gradient centrifugation, the accumulation of lipid raft-associated proteins including TLR4, EGFR, and GFRα1 after the ligand stimulation was decreased in FABP7-KO astrocytes. The activation of MAPKs (ERK, p38, JNK) and NF-kB was also decreased in FABP7-KO astrocytes compared with WT as examined by western blot using anti-phosphorylated p-MAPKs and (p)-IkB antibodies. Interestingly, in qPCR analysis, the expression of caveolin-1, the main protein component of caveolae, was significantly decreased at transcriptional level in FABP7-KO astrocytes. Deletion analysis of Cav-1 gene by using luciferase reporter assay revealed that FABP7-dependent promoter was located in an approximately 200bp upstream from the start codon. [Discussion]It is suggested that FABP7 may control the lipid raft (caveolae) formation thorough regulating the expression of caveolin-1, thereby being involved in the response of astrocytes to various external stimuli.

Disclosures: Y. Kagawa: None. **M. Ebrahimi:** None. **K. Sharifi:** None. **A. Islam:** None. **Y. Yasumoto:** None. **H. Miyazaki:** None. **S. Kawamura:** None. **Y. Yamamoto:** None. **T. Sawada:** None. **N. Tokuda:** None. **Y. Owada:** None.**Poster**

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.01/G35

Topic: B.11. Glia-Neuron Interactions

Support: BBSRC CASE studentship

GlaxoSmithKline funding

Title: Inflammatory pain behaviour is attenuated in GPR84 knock-out mice

Authors: *L. C. NICOL[1], C. GENTRY[1], D. MCINNERNEY[1], J. B. DAVIS[2], M. MALCANGIO[1], S. B. MCMAHON[1];
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Abstract: GPR84 is an orphan G-protein coupled receptor found in a range of immune cells. Under inflammatory conditions its expression is markedly induced in monocytes/macrophages and microglia and thus has been suggested to have a role in immunological and neuroinflammatory processes (Wang et al., 2006; Bouchard et al., 2007). Since accumulating evidence supports a critical role for neuronal-immune interactions in chronic pain generation, we studied the role of this receptor in inflammatory pain modulation.
GPR84 wild-type (WT) and knock-out (KO) C57BL/6 mice were generated and provided by GlaxoSmithKline. To examine the role of GPR84 in spinal microglia, mechanical, thermal or cold paw withdrawal thresholds of KO and WT mice were monitored over a 14 day period following either Complete Freund's Adjuvant (CFA) or lipopolysaccharide (LPS) administration. At the endpoint of the experiment, perfusion-fixed lumbar spinal cords were taken for immunohistochemical analysis of microglial markers Iba-1 and p-38 MAPK.
Following intraplantar CFA administration, GPR84 KO mice showed a 58% and 51% attenuation in the development of mechanical and cold hyperalgesia respectively, compared to WT littermate controls, which was significant. Interestingly, tactile mechanical thresholds dropped to a similar extent in both genotypes. In addition, CFA treatment caused significantly increased Iba1 and p-38 MAPK immunoreactivity in the ipsilateral dorsal horn of the spinal cord in both WT and KO compared to sham animals, with no difference between genotypes. Intrathecal LPS elicited significant mechanical allodynia 1 hr after injection in both genotypes. Iba1 and p-38 MAPK immunoreactivity in the lumbar spinal cord of WT or KO showed a similar bilateral increase compared to sham animals.
We conclude that GPR84 plays a role in the establishment of inflammatory pain. These data suggest that microglia do not contribute to the behavioural phenotype of GPR84 KO mice, as

following CFA or LPS there was no difference in microgliosis between genotypes. Furthermore, both genotypes exhibited a similar decrease in tactile mechanical paw withdrawal thresholds post intrathecal LPS, which is a CNS inflammatory model that selectively examines the microglia role. We therefore postulate that GPR84 expressed on peripheral macrophages may account for the observed attenuation of mechanical hyperalgesia of GPR84 KOs in both the CFA and LPS models.

Disclosures: L.C. Nicol: None. **C. Gentry:** None. **D. Mcinnerney:** None. **J.B. Davis:** None. **M. Malcangio:** None. **S.B. McMahon:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.02/G36

Topic: B.11. Glia-Neuron Interactions

Support: JSPS KAKENHI 10J00463

JSPS KAKENHI 22221004

JSPS KAKENHI 23657116

Kyushu University Global COE Program (Cell-fate Decision Function and Dysfunction in Homeostasis)

Title: Fosb gene products regulate expression of C5ar1 and C5l2 genes and microglial activation

Authors: *H. NOMARU, K. SAKUMI, D. TSUCHIMOTO, Y. NAKABEPPU;
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Abstract: The Fosb gene encodes subunits of the activator protein-1 (AP-1) complex, and is involved in the regulation of gene expression in multiple signaling pathways. Among the Jun and Fos gene families, Fosb forms two types of mature mRNA by alternative splicing, Fosb and ΔFosb, that encode full length FOSB and truncated ΔFOSB proteins. ΔFOSB lacks the C-terminal transactivation domain of FOSB. FOSB and ΔFOSB act as transcriptional regulators yet little is known about their downstream target genes. We already reported Fosb-null mice exhibit depressive-like behaviors and increase anxiety-like responses. In addition, these mice displayed impaired neurogenesis in the adult hippocampus and spontaneous epilepsy. These results strongly suggest that Fosb gene products have important roles in prevention of neurological and psychiatric disorders. Extensive research of their function in the brain has primarily been focused

on neurons, while that in glial cells remains to be explored. In this study, we found that microglial cells express high levels of Fosb and ΔFosb mRNAs as seen in hippocampal neurons, and identified genes whose expression depends on Fosb gene products in microglia. Using microarray analysis, we found that expression levels of 6 genes are significantly altered, with a fold change ≥1.5 (ANOVA; $p<0.05$), between primary microglia isolated from wild-type and Fosb-null brains. Of the 6 genes, we further investigated C5ar1 and C5l2, both of which encode receptors for the complement anaphylatoxin, C5a, an inflammatory mediator of the innate immune system. In Fosb-null microglia, chemotactic responsiviness tward C5a as well as levels of C5ar1 and C5l2 mRNAs were significantly decreased compared with wild type. We further investigated effect of disruption of Fosb gene on kainic acid (KA)-induced excitotoxicity in the hippocampus. We found Fosb-null mice exhibited an increased resistance to KA-induced seizure. Twenty four hours after KA administration, expression of both C5ar1 mRNA and C5ar1 protein was significantly increased in wild-type but not Fosb-null hippocampus. While level of C5l2 mRNA was not increased after KA administration, the basal level of C5l2 mRNA in Fosb-null hippocampus was significantly lower than that in wild type. Furthermore, levels of mRNAs for IL-6 and TNF-α in wild-type but not Fosb-null hippocampus were significantly increased accompanied by microglial activation, especially in CA3 region after KA administration. We thus concluded that Fosb gene products are indispensable to induce neuroinflammation through regulating expression of C5ar1 and C5l2 genes in microglia.

Disclosures: H. Nomaru: None. **K. Sakumi:** None. **D. Tsuchimoto:** None. **Y. Nakabeppu:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.03/G37

Topic: B.11. Glia-Neuron Interactions

Support: NHRI Grant NP-102-PP-09

NHRI Grant EX101-9940NI

NSC Grant 101-2321-B-010 -022

Title: Role of aryl hydrocarbon receptor in LPS-induced microglial activation

Authors: *F.-S. SHIE[1], C.-H. LIN[2], P.-C. HSU[3], Y.-P. YEH[3], Y.-Y. SUN[4], C.-Y. KUAN[4], J.-H. ZHUO[5], Y.-H. LEE[3];

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Abstract: Microglia become activated triggering various immune responses during the pathogenesis in many neurodegenerative diseases. Microglial activation is a double-edged sword and the resulting consequences of microglial activation can be beneficial or detrimental. However, the underlying mechanisms are not understood fully. Here, we reported that lipopolysaccharide (LPS) promoted activation of aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, and increased expression of AhR in primary microglia. LPS-induced expression of AhR can be diminished by pretreatment of ERK1/2 inhibitor, U0126, suggesting that ERK1/2 signaling may be involved. Importantly, small interfering RNA against AhR significantly attenuated LPS-induced microglial immune responses as evidenced by reduced expressions of inducible nitric oxide synthases (iNOS) and cyclooxygenase-2 (COX-2) and by suppressed secretion of pro-inflammatory cytokines, including tumor necrosis factor (TNFα) and interleukin-6 (IL-6). Formylindolo[3,2-*b*]carbazole (FICZ), an endogenous AhR ligand, also activated AhR in primary microglia. However, the LPS-induced microglial immune responses were alleviated by the co-treatment of FICZ. Taken together, our results suggest that AhR may function to aggravate LPS-induced microglial activation, while activation of AhR may counteract the deteriorating effects in a ligand-dependent manner. Therefore, further understanding the multi-functional aspects of microglial AhR can depict the regulatory machinery of microglial activation and will confer molecular basis for developing the treatment of the diseases.

Disclosures: F. Shie: None. **Y. Lee:** None. **C. Lin:** None. **P. Hsu:** None. **Y. Yeh:** None. **Y. Sun:** None. **C. Kuan:** None. **J. Zhuo:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.04/G38

Topic: B.11. Glia-Neuron Interactions

Title: Dopamine attenuates LPS-induced cytokine expression in mouse microglial BV-2 cells

Authors: *Y. SUGINO, Y. YOSHIOKA, Y. WADA, A. YAMAMURO, Y. ISHIMARU, S. MAEDA;
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Abstract:
The proinflammatory cytokines produced by activated microglia have been proposed to play a pathogenetic role in Parkinson's disease. The most important neuropathological alteration in Parkinson's disease is the loss of dopaminergic neurons in the substantia nigra, followed by a severe depletion of dopamine in the striatum. From these observations, we hypothesize that dopamine may negatively regulate cytokine production by activated microglia. To reveal this hypothesis, in this study, we investigated the effect of dopamine on LPS-induced mRNA expression of cytokines in mouse microglial cell line BV-2. The mRNA levels of IL-1β, IL-6 and TNF-α were determined by real-time RT-PCR, and the protein levels of NF-κB p65 and IκBα were determined by Western blotting. In BV-2 cells, LPS increased mRNA levels of IL-1β, IL-6 and TNF-α in a concentration-dependent manner. Pretreatment with dopamine (1-30 μM) for 24 h concentration dependently attenuated the LPS-induced mRNA expression of these cytokines. Neither SCH23390 nor sulpiride, D_1-like and D_2-like dopamine receptor antagonists, respectively, affected the attenuation of LPS-induced mRNA expression of cytokines by dopamine. In addition, pretreatment with CY208-203 or bromocriptine, D_1-like and D_2-like dopamine receptor agonist, respectively, did not affect LPS-induced mRNA expression of cytokines. N-Acetylcysteine (NAC), a free radical scavenger, inhibited the attenuation of LPS-induced mRNA expression of cytokines by dopamine. On the other hand, hypoxanthine/xanthine oxidase, a super oxide generating system, did not affect LPS-induced mRNA expression of cytokines. Dopamine concentration-dependently increased the level of quinoproteins, and the increase was inhibited by NAC. LPS increased the nuclear levels of NF-κB p65, and decreased the levels of IκBα in cytosol. The increase in the nuclear levels of NF-κB p65 was attenuated by dopamine. On the other hand, dopamine did not affect the LPS-induced decrease of IκBα. NAC inhibited the attenuation of LPS-induce increase in the nuclear levels of NF-κB p65 by dopamine. These results suggest that dopamine attenuates LPS-induced expression of cytokines by inhibiting the nuclear translocation of NF-κB p65 through the formation of quinoprotein in BV-2 cells. These findings indicate that the decrease of dopamine may enhance cytokine production by activated microglia and result in progression of Parkinson’s disease.

Disclosures: Y. Sugino: None. **Y. Yoshioka:** None. **Y. Wada:** None. **A. Yamamuro:** None. **Y. Ishimaru:** None. **S. Maeda:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.05/G39

Topic: B.11. Glia-Neuron Interactions

Support: NCCAM, ODS, NCI Grant P50AT006273

NIA Grant P01 AG018357

Title: Up-regulation of secretory phospholipases A2-group V in microglial cells: role of ERK1/2 in IFN-γ and LPS induced inflammatory signaling pathways

Authors: *Y. ZONG, J. JIANG, D. Y. CHUANG, A. SIMONYI, G. Y. SUN;
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Abstract: Secretory phospholipases A2 (sPLA2s) belong to a superfamily of enzymes responsible for hydrolysis of acyl ester at the *sn-2* position of glycerophospholipds. Among the more than 12 isoforms of sPLA2s in mammals, many studies have focused on group IIA because of its potential role in inflammatory responses and its ability to induce neuronal death via apoptosis. While sPLA2-IIA is expressed in rat astrocytes, our earlier studies failed to find it in rat or murine immortalized microglial cells. However, sPLA2-V is found constitutively present in rat and murine microglial cells, and is up-regulated upon treatment with pro-inflammatory cytokines (mixture of TNF-α, IL-1β and IFN-γ) as well as endotoxin (lipopolysaccharides, LPS). Our goal of this study is to elucidate the mechanism for regulation of sPLA2-V expression in microglial cells. Unlike sPLA2-IIA, which is normally up-regulated through the NF-κB pathway without the requirement of IFN-γ, up-regulation of sPLA2-V expression in microglial cells can be induced by exposing cells to IFN-γ alone; and IFN-γ can enhance sPLA2-V up-regulation by cytokines and LPS. Further studies led to results showing that besides the JAK-STAT1 pathway, IFN-γ can activate several other signaling pathways in microglial cells. For example, IFN-γ can activate the MAPK pathway leading to ERK1/2 phosphorylation, and in turn, p-ERK is important for production of reactive oxygen species (ROS) through phosphorylation of cytosolic subunits of NADPH oxidase. In addition, IFN-γ-induced ERK1/2 phosphorylation could enhance the NF-κB pathway through phosphorylation of IKK. Taken together, our data together with other studies demonstrate the pro-inflammatory role of sPLA2-V in microglial cells and its up-regulation through a signaling pathway involving IFN-γ, ERK1/2, IKK and ROS production. Understanding this oxidative pathway is important for development of therapeutic targets to suppress microglial inflammatory responses and interaction with neuronal behavior.

Disclosures: Y. Zong: None. **J. Jiang:** None. **D.Y. Chuang:** None. **A. Simonyi:** None. **G.Y. Sun:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.06/G40

Topic: B.11. Glia-Neuron Interactions

Support: NIH Grant 5P01 AG018357

NIH Grant P50AT006273

Title: Role of cytosolic phospholipase A2 in lipopolysaccharide- and interferon gamma-induced inflammatory responses in microglial cells

Authors: *D. Y. CHUANG[1], Y. ZONG[1], J. JIANG[2], A. SIMONYI[1,2], Z. GU[1,3], G. Y. SUN[1,2,3]; [1]Interdisciplinary Neurosci. Program, [2]Dept. of Biochem., [3]Dept. of Pathology and Anatom. Sciences, Ctr. for Translational Neurosci., Univ. of Missouri, Columbia, MO

Abstract: Oxidative stress and inflammatory responses have been shown to contribute to the pathophysiology of numerous neurological diseases, including Alzheimer's disease, Parkinson's disease, acute stroke, and infections of the brain. There is increasing evidence for the role of microglial cells in mediating the oxidative and inflammatory responses and propagating damage to other cell types in the brain. Microglial cells readily respond to endotoxin (lipopolysaccharide, LPS) and cytokines and upon activation, they further generate additional pro-inflammatory cytokines, as well as reactive oxygen species (ROS) and nitric oxide (NO). In our recent studies, we have demonstrated the important role of extracellular signal-regulated kinase (ERK1/2) and reactive oxygen species (ROS) in transducing inflammatory signals that leads to iNOS expression in microglia after stimulation with LPS or interferon-gamma (IFNg). Microglia also express relatively high levels of cytosolic phospholipase A2 (cPLA2), an enzyme known for regulation of membrane phospholipid homeostasis and release of arachidonic acid (AA) for synthesis of eicosanoids. Activity of cPLA2 is highly determined by the phosphorylation state of the Ser505 residue, largely through the mitogen-activated protein kinases (MAPKs), namely ERK1/2 and p38. However, whether cPLA2 downstream plays a role in modulating microglial oxidative and inflammatory responses has not been investigated in detail. In this study, we observed evidence for a link between cPLA2 and signaling pathways for LPS/IFNg-induced NO and ROS production in microglial cells. With the immortalized mouse microglia BV-2 cell line, inducible nitric oxide (iNOS) expression and its subsequent NO production due to treatment with LPS or IFNg were both dose-dependently inhibited by AACOCF3, an inhibitor of cPLA2 and iPLA2. AACOCF3 exerted a much greater inhibitory effect on LPS stimulated NO as compared

to IFNg stimulated NO. A similar trend for effect of AACOCF3 is observed in ROS production, which has been shown in previous study to generate from the active NADPH oxidase complex downstream of ERK1/2 activation (Chuang et al., J Neuroinflammation, 2013). Interestingly, inhibition of pERK1/2 with U0126, a MEK1/2 inhibitor, shows reciprocal effect, i.e. U0126 was more potent in inhibiting IFNg-stimulated NO/ROS than LPS-stimulated NO/ROS. Taken together, results show that while cPLA2 is shown to play a role in modulating inflammatory responses in microglia cells, its regulation may be mediated by different receptor activation pathways. These cross-talk mechanisms will be the center of investigation in future studies.

Disclosures: D.Y. Chuang: None. **Y. Zong:** None. **J. Jiang:** None. **A. Simonyi:** None. **G.Y. Sun:** None. **Z. Gu:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.07/G41

Topic: B.11. Glia-Neuron Interactions

Support: FCT (PTDC/EBB-EBI/115810/2009)

DARPA (09-68-ESR-FP-010)

Title: Adenosine A2A receptors control the metabolic changes associated with microglia activation as revealed by NMR isotopomeric analysis

Authors: *C. LEMOS[1], G. CRISTÓVÃO[2], I. JARAK[2], R. A. CUNHA[2], C. GOMES[2], R. A. CARVALHO[2];
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Abstract: Microglia cells play a critical role in brain homeostasis. Both physiological changes (e.g. synaptic activity) or disease-associated abnormalities (e.g. bacterial or proteinaceous antigens) trigger a metabolic adaptation of microglia to sustain adequate reactivity, namely proliferation. The modulation of microglia proliferation is of potential therapeutic interest to manage brain disorders, as heralded by the ability of adenosine A2A receptor (A2AR) antagonists to control microglia proliferation (Gomes et al., 2013, J Neuroinflamm 10:16) and alleviate brain diseases (Gomes et al., 2011, Biochem Bipohys Acta 1808:1380). We now tested if A2AR control the mandatory metabolic adaptation that enables microglia to respond to "danger" signals.
We used 1H- and 13C- NMR spectroscopy to characterize some key metabolic features

(glycolytic activity, assessed by lactate/alanine ratio, and coupling between glycolytic and Krebs cycle fluxes, gauged as the 13C incorporation rate into glutamate) of non-activated and activated (with lipopolysaccharide, LPS, 100 ng/mL) N9 microglia cells, in the absence or presence of the selective A2AR antagonist, SCH 58261 (50 nM, for 48h).
Non-activated cells exhibited a low lactate/alanine ratio and high 13C incorporation rate into glutamate. LPS increased the lactate/alanine ratio (associated with increased glycolysis and reduction of alanine content) and abolished the 13C incorporation into glutamate. A2AR blockade did not affect the metabolic profile of non-activated cells, but modulated LPS-induced changes in a time-dependent manner: the initial counteraction of glycolytic fluxes (2-6h) was followed by a return to basal levels by the end of the incubation period (48h); this time-dependent modulation was also noticeable by an increase of the incorporation of 13C in glutamate in the steady state isotopomer analysis performed in cell extracts.
These findings indicate that LPS determines a metabolic reshaping of microglia cells to cope with LPS-induced proliferation. A2AR blockade, which prevents LPS-induced proliferation (Gomes et al., 2013), also impacts on the metabolic adaptation of activated microglia, optimizing the coupling between glycolysis and Krebs cycle fluxes.

Disclosures: C. Lemos: None. **G. Cristóvão:** None. **I. Jarak:** None. **R.A. Cunha:** None. **C. Gomes:** None. **R.A. Carvalho:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.08/G42

Topic: B.11. Glia-Neuron Interactions

Title: Prostaglandin E2 exerts anti-inflammatory effects by inhibiting microglial production of superoxide through a novel pathway

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Abstract: Prostaglandin E2 (PGE2) is a well-known pro-inflammatory mediator, but recent studies indicate that PGE2 also has anti-inflammatory functions. PGE2 exerts its actions by binding to one or several of its receptors (EP1 to EP4) coupled to different G proteins and linked to different second messengers. The anti-inflammatory effects of PGE2 are mediated by EP2 and EP4 receptors, which trigger cAMP production, leading to protein kinase A (PKA) activation.

However, several studies suggest that PKA may not be the only pathway mediating the anti-inflammatory effect of PGE2. The main purpose of this study was to search for the novel PKA-independent pathways of PGE2. Unlike previous studies that used pharmacological concentrations of PGE2 (100 nM to 10 microM), we used physiological concentrations of PGE2 (<10 nM) for studying its anti-inflammatory effect in LPS-treated rodent mixed glia cultures. Our results show that PGE2 inhibited superoxide production in microglia with an IC50 of 0.1 nM which is 10 to 100-fold lower than the binding affinity of EP2 and EP4 receptors. Meanwhile, agonists for all the EP receptors mimicked this inhibition with similar affinities. PGE2-elicited inhibition of superoxide production was not affect in EP2-deficient microglia,indicating the existence of an EP2 receptor-independent mechanism. Pharmacophore analysis of the structure of these EP receptor agonists revealed a common binding site shared by these agonists which maybe associated with superoxide production. Further mechanistic studies revealed that PGE2-elicited reduction of LPS-induced superoxide production was mediated through the inhibition of the key microglial superoxide-producing enzyme, phagocyte NADPH oxidase (PHOX), via binding to the catalytic subunit of PHOX gp91^{phox}. Furthermore, this novel superoxide inhibitory effect is associated with PGE2-induced reduction in TNFa production in LPS-treated microglia. The evidence came from studies showing that 1) inhibition of TNFa production can be achieved in a concentration of PGE2 (10 nM) that did not increase microglial cAMP levels, 2) inhibitors of adenylate cyclase or PKA failed to reverse PGE2-elicited suppression of TNFa, and 3) PGE2-elicited inhibition of TNFa was not observed in microglia deficient in PHOX. Taken together, or study revealed a PHOX dependent and cAMP/PKA-independent novel pathway mediating the anti-inflammatory effects of physiological concentrations of PGE2.

Disclosures: S. Chen: None. **E. Oyarzabal:** None. **J. Hong:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.09/G43

Topic: B.11. Glia-Neuron Interactions

Support: CIHR

FRSQ

CNRS

Title: Inhibition of phosphoinositide-dependent P2X4 receptor channel by Gq-coupled P2Y6 receptor in microglia

Authors: *L.-P. BERNIER[1,2], A. ASE[2], E. BOUE-GRABOT[3], P. SEGUELA[2];
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Abstract: ATP-gated P2X4 receptor channels expressed in spinal microglia actively participate in central sensitization, making their functional regulation a key process in chronic pain pathologies. P2Y6 metabotropic Gq-coupled receptors, also expressed in microglia, are involved in the initial response to nerve injury, triggering phagocytosis upon activation by UDP. It has been reported recently that expression of both P2X4 and P2Y6 is upregulated in activated microglia following nerve injury. We show here, in resting as well as LPS-activated primary microglia, that P2Y6 decreases P2X4-mediated calcium entry and inhibits the dilation of P2X4 channels into a large-conductance pore measured with a YO-PRO-1 uptake assay. Furthermore, P2Y6 activation modulates the ATP-dependent migration of microglia, a process likely involved in their shift from migratory to phagocytic phenotype. Reconstituting the P2X4-P2Y6 interaction in recombinant systems shows that P2Y6 activation decreases P2X4 current amplitude, activation and desensitization rates, and reduces P2X4 channel permeability to the large cation NMDG+. Phospholipase C-mediated hydrolysis of the phosphoinositide PI(4,5)P2, a necessary cofactor for P2X4 channel function, underlies this inhibitory crosstalk. As extracellular levels of both ATP and UDP are increased in the spinal cord following nerve injury, the control of P2X4 activity by P2Y6 might play a critical role in regulating neuropathic pain-inducing microglial responses.

Disclosures: L. Bernier: None. **P. Seguela:** None. **A. Ase:** None. **E. Boue-Grabot:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.10/G44

Topic: B.11. Glia-Neuron Interactions

Support: NSERC Discovery Grant

Canadian Foundation for Innovation Grant

Title: Involvement of microglial P2X7 receptors in morphine analgesic tolerance is mediated by mu-opioid receptor signalling

Authors: H. L. LEDUC-PESSAH[1,2,3], C. FAN[1,2,3], *T. TRANG[1,2,3];
[1]Comparative Biol. & Exptl. Med., [2]Physiol. & Pharmacol., [3]Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Morphine is indispensable in the treatment of acute and chronic pain. However, its use is limited by the development of analgesic tolerance, such that higher and more frequent doses are necessary for achieving the same level of pain control. In rats, we found that the development of morphine tolerance correlated with an increase in spinal expression of ATP-gated P2X7 receptors (P2X7R). This increase was restricted to microglia residing in the spinal dorsal horn, and pharmacologically blocking P2X7Rs with the selective P2X7R antagonist, A740003, attenuated the development of tolerance but did not reverse established tolerance. Likewise, in microglial BV2 cells morphine treatment caused a dose dependent upregulation of P2X7Rs that was suppressed by the mu-receptor antagonist, CTAP. By contrast, activating mu receptors with DAMGO markedly upregulated total P2X7R protein expression in the microglia. Although P2X7R mRNA levels were not affected by morphine treatment, we detected an increase in cell surface expression of P2X7Rs that was concomitant with enhanced BzATP-evoked influx of extracellular Ca2+. Taken together, our findings suggest that mu-opioid receptor signaling critically regulates expression of microglial P2X7Rs involved in the development of morphine analgesic tolerance.

Disclosures: H.L. Leduc-Pessah: None. **C. Fan:** None. **T. Trang:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.11/G45

Topic: B.11. Glia-Neuron Interactions

Support: NIH NS041421

Dept. of Veterans Affairs

Title: Activation of poly(ADP-ribose) polymerase-1 in males and females: Comparisons using PAR immunostaining and a PARP activity assay

Authors: *Y. CHEN[1,2], S. WON[1], C. HEFNER[1], Y. SHEN[1], Y. XU[2], R. SWANSON[1];
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Abstract: Poly(ADP-ribose)polymerase-1 (PARP-1) is an abundant nuclear enzyme that consumes cytosolic NAD^+ to form poly(ADP-ribose) polymers (abbreviated as PAR) on histones and other proteins in the vicinity of single strand DNA breaks. These breaks occur normally in the course of gene transcription, and occur pathologically in response to oxidative stress, excitotoxicity, and ischemia reperfusion. PAR formation provides a scaffolding for protein complexes involved in single strand DNA repair, but extensive PAR formation can deplete cytosolic NAD^+ concentrations and precipitate cell death. PARP-1 activation also has signaling and regulatory effects in cells, and in particular it facilitates NF-kB -driven gene transcription during inflammatory responses. The effect of PARP inhibitors on PARP-1 - induced cell death and inflammatory responses are strikingly different in males and females, for reasons that remain obscure. A key question is whether these gender-specific effects of PARP inhibitors occur downstream of PARP activation, or whether instead PARP activation is inherently different in male and females under comparable stimuli. For technical reasons this has been difficult to resolve. One way to evaluate PARP-1 activity in brain sections is by Western blots or immunostaining for the PARP-1 product, PAR. However, the amount of PAR present at any given time may be an unreliable indicator of enzyme activity, because PAR levels are also affected by the rate of PAR degradation. For this reason we adapted a PARP activity assay to address this issue. The method employs biotin-labeled NAD^+, which is then trapped in both PAR and PAR degradation products, and permits histochemical evaluation of PARP activity in both cultured cells and ex vivo brain sections. Our studies show the feasibility of comparing these two techniques in both cell culture and ex vivo brain sections, which indicate that the effects PARP inhibitors on the NF-kB activity may be different at short and long term at points following inflammatory stimuli.

Disclosures: Y. Chen: None. **S. Won:** None. **C. Hefner:** None. **Y. Shen:** None. **Y. Xu:** None. **R. Swanson:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.12/G46

Topic: B.11. Glia-Neuron Interactions

Support: Heart & Stroke Foundation of Canada

Canadian Institutes for Health Research

Title: Activation of neuronal NMDA receptors triggers rapid microglial process outgrowth

Authors: *L. DISSING-OLESEN, J. LEDUE, R. RUNGTA, H. CHOI, B. MACVICAR; Brain Res. Centre, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Microglia exhibit motile processes that survey surrounding synapses by contacting dendritic spines in response to unknown signals. Microglia extend processes in response to ATP and activation of neuronal NMDA receptors (NMDAR) has been demonstrated to trigger opening of the ATP releasing channel; pannexin 1 (Panx1). Therefore we tested the hypothesis that activation of neuronal NMDAR triggers ATP efflux through opening of Panx1 channels to signal to microglia. Here we show that activation of neuronal NMDAR indeed triggers ATP-mediated microglial process outgrowth but surprisingly the release of ATP occurred independently of Panx1 opening.

We used two-photon scanning microscopy of EGFP+ve microglia in acute hippocampal brain slices to confirmed that extracellular ATP triggered a characteristic microglial process outgrowth that reversed when ATP was removed or when purinergic receptors were blocked. Brief NMDAR activation in the CA1 stratum radiatum triggered a similar microglial process outgrowth. This microglia outgrowth in response to NMDA was reversible and repeatable, demonstrating that the microglial response was not due to permanent excitotoxic damage. We also observed that the process outgrowth was more pronounced and occurred more reliably following multiple NMDA applications. The underlying mechanism(s) for this 'priming effect' is under investigation.

The microglial response was selective to NMDAR stimulation as it was blocked by APV, the selective NMDAR antagonist, but still occurred in the presence of CNQX and TTX to block AMPA receptors and Na+ channels, respectively. The microglia were responding to the release of ATP triggered by NMDA application not NMDA itself because microglia process outgrowth was abolished by purinergic receptor blockade. Surprisingly, the commonly used blocker of pannexin and connexin channels, carbenoxolone did not block NMDA-induced microglia process outgrowth although as a control we showed carbenoxolone did block dye flux through connexin gap junctions. To further validate this result, we established a modified fixation protocol that allowed us to fix the whole brain slice and preserve the microglia morphology at the time of fixation. Immunohistochemical labeling of microglia in tissue from Panx1-deficient mice subjected to multiple NMDA applications confirmed that outgrowth occurred independent of Panx1. Thus the mechanism(s) of NMDA-mediated ATP release is currently under investigation.

Disclosures: L. Dissing-Olesen: None. **J. LeDue:** None. **R. Rungta:** None. **H. Choi:** None. **B. MacVicar:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.13/G47

Topic: B.11. Glia-Neuron Interactions

Support: GUMC start-up funds to KMZ

Title: Characterization of cortical and sub-cortical glia following toll-like receptor stimulation

Authors: *C. WINLAND[1], S. G. DANIELE[2], A. G. EDWARDS[2], K. MAGUIRE-ZEISS[2]; [1]Georgetown Univ., Fairfax, VA; [2]Georgetown Univ., Washington, DC

Abstract: Neuroinflammation coincides with the progression of neurodegenerative diseases such as Alzheimer's, Parkinson's, and ALS. It is well established that activated microglia and astrocytes release neurotoxic molecules including interleukin-1 beta (IL-1β), tumor necrosis factor (TNFα), and nitric oxide (NO). The release of these pro-inflammatory molecules may exacerbate neuronal cell loss through enhanced oxidative stress and/or cytokine-mediated cell death. However, the difference between regionally distinct microglia and astrocyte responses to brain-relevant stimuli are not well characterized. Therefore, the present study compared release of pro-inflammatory molecules from microglia and astrocytes stimulated with toll-like receptor ligands and the Parkinson's disease proteinα-synuclein. Primary microglia and astrocytes were cultured from cortical or subcortical areas of C57/Bl6 mice. Following stimulation cells, lysates, and media were harvested and analyzed for proinflammatory molecules. There were no cell type or regional differences in glial responses to TNFαproduction following stimulation with a TLR2 or TLR4 ligand. However, immunocytochemistry for Iba1 revealed subtle morphological differences between cortical and subcortical microglia. Interestingly, oligomeric α-synuclein elicited greater TNFαrelease from subcortical microglia when compared to microglia cultured from cortical areas. These results demonstrate differential regional activation of microglia to a Parkinson's disease relevant protein, which supports the need for more studies to elucidate whether this region specific activation plays a role in Parkinson's disease pathogenesis.

Disclosures: C. Winland: None. **S.G. Daniele:** None. **A.G. Edwards:** None. **K. Maguire-Zeiss:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.14/G48

Topic: B.11. Glia-Neuron Interactions

Title: Involvement of MAP kinase cascade in M-CSF-triggered microglial proliferation in transected rat facial nucleus

Authors: *S. YAMAMOTO[1], S. KOHSAKA[2], K. NAKAJIMA[1,2];
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Abstract: Transection of rat facial nerve leads to an increase of microglial cell number in the ipsilateral facial nucleus. In the previous study, we demonstrated that up-regulated macrophage-colony stimulating factor (M-CSF) in the transected facial nucleus triggers the induction of cFms (receptor for M-CSF), proliferating cell nuclear antigen (PCNA) and cell cycle-associated proteins, including cyclins, cyclin-dependent protein kinases (Cdks) and Cdk inhibitors (CdkIs) in microglia and causes the microglia to divide. However, the signaling mechanism of M-CSF-triggered microglial proliferation remains to be elucidated. In the present study, we analyzed the signaling cascade downstream of cFms. Experiments in vitro using a cFms inhibitor indicated that M-CSF-cFms signaling leads to upregulation of the levels of cFms, PCNA, cyclin A and cyclin D in microglia. The role of cyclin A/Cdk2 activity in M-CSF-dependent microglial proliferation was ascertained by using the specific inhibitor purvalanol A. C-Jun N-terminal kinase (JNK) was suggested to be associated with M-CSF-dependent induction of cyclins and PCNA, while p38 was associated with cFms induction. Both JNK and p38 were proved to be phosphorylated by stimulation with M-CSF, and were significantly suppressed by pretreatment with cFms inhibitor. Furthermore, we demonstrated that mitogen activated protein kinase activated protein kinase-2 (MAPKAPK-2), cyclic AMP responsive element binding protein (CREB) and mitogen-and stress-activated protein kinase-1 (MSK1) are activated in M-CSF stimulated microglia. These signaling molecules are thought to be located at downstream of JNK/p38 because their phosphorylations were suppressed in the presence of JNK/p38 inhibitors. Our results indicated that MAPKAPK-2, CREB and MSK1 are involved in JNK/p38 cascade downstream of cFms in M-CSF-stimulated microglia.

Disclosures: S. Yamamoto: None. **S. Kohsaka:** None. **K. Nakajima:** None.

Poster

522. **Microglia: Signaling**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 522.15/G49

Topic: B.11. Glia-Neuron Interactions

Support: the Mid-career Researcher Program through the National Research Foundation of Korea (NRF) grant funded by the MEST (2011-0028319)

Title: Osteopontin expression by dying neurons in oxygen-glucose-deprived hippocampal slice cultures

Authors: T.-R. RIEW[1], Y.-J. SHIN[1], J.-H. PARK[1], H. KIM[2], *M.-Y. LEE[1];
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Abstract: Osteopontin (OPN), an adhesive glycoprotein, has recently been proposed to act as an opsonin that facilitated phagocytosis of neuronal debris by macrophages in the ischemic brain. Here, the present study was aimed to gain further insight into the relationship between OPN induction and neuronal cell death using organotypic hippocampal slice cultures subjected to ischemia-like oxygen-glucose deprivation (OGD). Double or triple-labeing study using markers of cell death such as propidium iodide uptake or the Tdt-dUTP terminal nick-end labeling (TUNEL) revealed revealed that OPN expression is induced exclusively in dying neurons and not in viable neurons after OGD. Immunoperoxidase electron microscopy revealed that labeled neurons whose cell bodies shrank and possessed condensed nuclei with small patches of chromatin clumping and many swollen organelles and vacuoles in the perikaryal region, displayed a mixture of necrotic- and apoptotic-like features after OGD. OPN Immunoreaction products in labeled neurons were diffusely localized throughout the nucleus and cytoplasm, and were also associated with the rough endoplasmic reticulum and mitochondria, while the reaction products in microglia were strictly localized to the Golgi apparatus, and not associated with other organelles or with the nucleus. In addition, phagocytic microglial cells were frequently located in a close vicinity of the labeled dying neurons, and contained labeled cell debris inside their cytoplasm, indicating that the labeled neurons fragmented into cell debris that was engulfed by microglia synthesizing and secreting OPN. Thus, our data suggest that OPN induction may be involved in the neuronal cell death and ongoing microglial phagocytosis of dead neurons during OGD and reperfusion in hippocampal slice cultures.
This study was supported by the Mid-career Researcher Program through the National Research Foundation of Korea (NRF) grant funded by the MEST (2011-0028319).

Disclosures: T. Riew: None. **M. Lee:** None. **Y. Shin:** None. **J. Park:** None. **H. Kim:** None.**Poster**

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.01/G50

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG027544

NIH Grant AG021982

Title: Genetic depletion of tau prevents cognitive impairment in type 1 diabetes-like mouse model

Authors: *D. BAGLIETTO-VARGAS[1], S. ABBONDANTE[1], C. J. RODRIGUEZ-ORTIZ[1,2], T. ESTRADA-HERNANDEZ[1], R. MEDEIROS[1], F. M. LAFERLA[1];
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Abstract: Growing evidence indicates a significant association between diabetes and Alzheimer disease (AD). Patients affected by diabetes show an increased risk of developing AD. However, the underlying molecular mechanisms connecting these two disorders are still not well understood. Here, we investigate the microtubule-associated protein tau (MAPT) as a new perspective on the association between AD and diabetes. To determine whether diabetes causes cognitive decline by tau dependent mechanism, we treated non-transgenic (Ntg) and tau knockout (tauKO) mice with streptozotocin (STZ), causing type 1 diabetes-like disease (T1D). Our study shows that tau is a fundamental mediator for T1D-like disease to induce cognitive impairment, and its dysregulation causes reduction in synaptic proteins levels and cognitive decline. Concomitantly, we demonstrate the novel finding that depletion of endogenous tau mitigates behavioral impairment and synaptic deficits induced in T1D-like mice. Therefore, our data reveal that tau is a key molecular factor necessary for T1D-like disease to induce cognitive decline, and represents a potential therapeutic target for diabetes and AD patients.

Disclosures: D. Baglietto-Vargas: None. **S. Abbondante:** None. **C.J. Rodriguez-Ortiz:** None. **T. Estrada-Hernandez:** None. **R. Medeiros:** None. **F.M. LaFerla:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.02/G51

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: The Wooten Foundation for Alzheimer's and Neurodegenerative Diseases Research

Title: Effects of differential exercise training on hippocampal function using a triple-transgenic mouse model of alzheimer's disease

Authors: *T. D. TRAN[1], Q. LU[2], S. BAREISS[3];
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Abstract: Alzheimer's disease (AD) is the primary cause of dementia and is characterized by severe brain, behavior, and cognitive dysfunctions that hinder everyday living for those affected. AD is the 6th leading cause of death in the United States and comprises 50-80% of dementia cases worldwide. In the past several years, a mouse model bearing three gene mutations of PS1-M146V (presenilin), APP-Swe (amyloid precursor), and tauP301L (tau) proteins (i.e., 3xTg model) has received considerable attention in elucidating pathology and neurocognitive deficits observed in AD. This mouse model has shown that significant cognitive decline and AD pathology usually occurs at 6 months of age. Research supports the notion that exercise maintains and improves cognitive function in aging, and remains the best non-pharmaceutical approach to combat cognitive decline and the progression of AD in humans (Blacker et al., 2007; Booth et al., 2002). Indeed, human and animal studies have shown that exercise may protect against cognitive decline and pathology (e.g., Cho et al., 2003; Dustman et al., 1984; Um et al., 2008). In this study, we examined whether chronic, differential exercise therapy affects hippocampal-based cognitive function using the spatial version of the Morris water maze. Our hypothesis is that increasing doses of exercise in 3xTg mice would attenuate the behavioral and pathological hallmarks of AD. There were six exercise groups: (1) sedentary controls (S-WT, S-3xTg); (2) mice that received exercise training once per week (1x-WT, 1x-3xTg); and (3) mice that received exercise training three times per week (3x-WT, 3x-3xTg). Prior to reaching 6 months of age, all exercise groups received 90 days of training and in each session they ran at a rate of 8.0 m/min for 60 min with a 5-min warm up and cool down. Two days afterwards, they received water maze testing that consisted of 4 days of acquisition (4 trials/day) and 1 day of memory retention with a single probe trial. The following day, brain tissue was harvested for examination of changes in biochemical and immunohistological markers of AD. Results showed that in general, mice that exercised had better recall performance, particularly when compared to sedentary 3xTg mice. Comparison of 3xTg groups showed that both exercise groups benefited from their respective training regimens in relation to the sedentary 3xTg group. Supported by The Wooten Foundation for Alzheimer's and Neurodegenerative Diseases Research grant to SB.

Disclosures: T.D. Tran: None. **Q. Lu:** None. **S. Bareiss:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.03/G52

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG037637

Glenn Foundation

William and Ella Owens Medical Research Foundation

NIH Grant R01 MH084315

Title: Dissecting the role of mTOR in Alzheimer's disease

Authors: *A. CACCAMO[1], M. F. LÓPEZ-ARANDA[2], A. J. SILVA[2], S. ODDO[1];
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Abstract: Accumulation of Aβ and tau is a critical event in Alzheimer's disease (AD); however, the molecular mechanisms leading to Aβ and tau accumulation remain unclear. Age is a major risk factor for AD, suggesting that molecular changes contributing to the aging process may facilitate Aβ and
tau accumulation. Here, we use multiple animal models and complementary genetic and pharmacological approaches to show that dysregulation of the mammalian target of rapamycin (mTOR) contribute to Aβ and tau accumulation. Specifically, we first show that there is an interrelation between Aβ and mTOR. Indeed, Aβ causes mTOR hyperactivity, which in turn further facilitates Aβ accumulation. Hyperactive mTOR also facilitate tau expression and phosphorylation. Toward this end, we also show that show that genetically increasing mTOR activity elevates
endogenous mouse tau levels and phosphorylation. Complementary to it, we further demonstrate that pharmacologically reducing mTOR signaling with rapamycin ameliorates tau pathology and the associated behavioral deficits in a mouse model overexpressing mutant human tau. In summary, we show that mTOR, a key kinase involved in controlling protein homeostasis, represent a link between Aβ and tau accumulation. Given the overwhelming evidence showing that reducing mTOR signaling increases lifespan and health span, the data presented here have profound clinical implications for aging and AD and provide the molecular basis for how aging may contribute to this insidious disorder. Additionally, these results provide pre-clinical data indicating that reducing mTOR signaling may be a valid therapeutic approach for AD.

Disclosures: A. Caccamo: None. **M.F. López-Aranda:** None. **A.J. Silva:** None. **S. Oddo:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.04/G53

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Mitochondrial O-GlcNAc modification in the brains of 5XFAD mice

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Abstract: Mitochondria are the essential organelles to supply cellular energy and maintain Ca^{2+} homeostasis. Dysfunctional mitochondria also have relevance in several human diseases, including Alzheimer's disease (AD). Mitochondrial dysfunctions occur in the early stages of AD. These changes are induced by amyloid beta (Aβ), one of the major hallmarks of AD. It is also well known that glucose metabolism is disrupted in the brains of AD patients. *O*-GlcNAcylation (O-GlcNAc) is similar to phosphorylation, which is a post-translational modification that occurs at the same sites as phosphorylation (Ser or Thr residues) and regulates diverse cellular processes. Since *O*-GlcNAc of proteins is regulated by intracellular glucose metabolism and AD patients show the reduced glucose metabolism in the brain, it is reasonable to predict the decrease of *O*-GlcNAc proteins in AD brains. However, it has not yet known the difference of O-GlcNAcylated proteins between normal subject brains and AD patient's brains. In the present study, we focused on Mitochondrial O-GlcNAcylated proteins in the brains of 5XFAD mice, which is AD animal model. Mitochondrial proteins were analyzed by using LC-MS/MS methods. We identified and quantified novel O-GlcNAcylated mitochondrial proteins in the brains of 5XFAD mice.

Disclosures: H. Choi: None. **C. Kim:** None. **M. Cha:** None. **I. Mook-Jung:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.05/G54

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Mesenchymal stem cells enhance autophagy and increase beta-amyloid clearance in Alzheimer's disease models

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Abstract:
Alzheimer's disease (AD) is characterized by neuronal loss and accumulation of abnormal amyloid β (Aβ) and tau proteins. Autophagy is a major degradation pathway for abnormal aggregated proteins and organelles that cause various neurodegenerative diseases. Current evidence suggests a central role for autophagy in AD pathogenesis, and dysfunction in the autophagic system may lead to Aβ accumulation.
Mesenchymal stem cells (MSCs) secrete various cytotropic factors that have neuroprotective effects through complex mechanisms such as modulation of neuroinflammation, enhancement of cell survival signals,and modulation of ubiquitinated proteins. In the present study, we investigated whether MSCs could enhance autophagy and thus exert a neuroprotective effect through modulation of Aβ clearance using in vitro and in vivo AD models. In Aβ-treated neuronal cells co-cultured with MSCs, MSCs increased cellular viability and enhanced LC-II expression compared with cells treated with Aβ only. Immunofluorescence analysis revealed that MSC treatment in Aβ-treated neuronal cells increased the LC3-positive autophagosomes that were co-localized with a lysosom. Additionally, cathepsin B mRNA expression, the major protease in lysosomes, was significantly increased in neuronal cells co-cultured with MSCs; treatment with chloroquine, a lysosomal inhibitor, significantly attenuated the increase in cell survival in cells co-cultured with MSCs. Ultrastructural analysis evealed that most autophagic vacuoles in Aβ-treated cells were not fused with lysosomes, whereas a large portion of autophagosomes were on joined with lysosomes in MSC-treated cells. Furthermore, MSC treatment markedly increased Aβ immunoreactivity co-localized within lysosomes and decreased intracellular Aβ levels compared with Aβ-treated cells. In an Aβ-treated animal model of AD, MSC administration significantly increased autophagosome induction, final maturation of late autophagic vacuoles, and fusion with lysosomes. Moreover, MSC administration significantly reduced the level of Aβ in the hippocampus, which was elevated in Aβ-treated mice, concomitant with increased survival of hippocampal neurons. Finally, MSC treatment up-regulated Beclin-1 expression in Aβ-treated cells and animals. These results suggest that MSC treatment significantly enhances autophagolysosome formation and clearance of Aβ in Aβ-treated cellular and animal models, which may lead to increased neuronal survival against Aβ toxicity. Modulation of the autophagy pathway to repair the damaged AD brain using MSCs would have a significant impact on future strategies for AD treatment.

Disclosures: J. Shin: None. **H. Park:** None. **H. Kim:** None. **S. Oh:** None. **P. Lee:** None.

Poster

523. Alzheimer's Disease: Interventions

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.06/G55

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: William C. Friday Chair Fund

Title: Enhancing the lysosomal degradation pathway promotes clearance of Aβ42 and α-synuclein in transgenic mouse models

Authors: U. S. IKONNE, J. HWANG, *B. A. BAHR;
Biotech. Res. and Training Ctr., Biotech Ctr. / William C. Friday Lab., Pembroke, NC

Abstract: Alzheimer's disease (AD) is the most frequent form of dementia and a key pathogenic factor is Aβ42 which accumulates as a result of over-production and/or inefficient clearance. The lysosomal enzyme cathepsin B (CatB) degrades Aβ42 by C-terminal truncation, lowering levels in mice expressing hAPP (Mueller-Steiner et al. 2006, *Neuron* 51:703; Butler et al. 2011, *PLoS One* 6:e20501; Wang et al. 2012, JBC 287:39834) and reducing both plaque load and behavioral and synaptic marker deficits. Accordingly, enhancing CatB is a promising drug discovery avenue for AD (Viswanathan et al. 2012, *ACS Med Chem Lett* 3:920), and studies suggest that lysosomal enhancement is also a potential strategy to reduce α-synuclein oligomers to treat Parkinson's disease (Lee et al. *J Neurosci* 24:1888). Here, the small-molecule CatB enhancing agent Z-Phe-Ala-diazomethylketone (PADK, 20 mg/kg/d i.p.) treated both 20-month APPswe/PS1ΔE9 mice and 8-month human α-synuclein A53T mice for 10-11 days, resulting
in a 4-8-fold increase in active CatB levels in brain samples as compared to vehicle-treated groups. In the APP-PS1 mice, the lysosomal enhancement was associated with a 62% reduction in Aβx-42 ELISA measures in hippocampal samples ($p<0.01$), and the 4-kDa band on 6E10 blots was also reduced by 60%. Synaptic marker deficits in the AD mice including GluR1, synapsin II, and NCAM180 exhibited
recovery in correspondence with the Aβ42 clearance. Recovered GluR1 levels correlated significantly with the extent of CatB enhancement. To test for α-synuclein clearance, PADK-treated A53T mice were assessed using TX-100 extractions. Similar to the effects on Aβ42, the PADK effect consisted of 50-70% declines in α-synuclein across spinal cord, brainstem, midbrain, and hippocampal samples ($p<0.01$). As in the AD tg mice, synaptic protection was also linked to the protein clearance. In fact, α-synuclein reduction across samples had a significant correlation with the extent of synaptic marker recovery. These
results indicate a single strategy can act against distinct protein accumulation events, promoting CatB-mediated protein clearance for disease-modifying treatments of early/progressive synaptic

decline and concomitant dementia. Contributing to the protective clearance, the CatB modulator PADK also has the ability to disaggregate Aβ42 oligomers (Zheng et al. 2012, *JBC* 287:6084), thus a role in oligomeric disaggregation may also lead to efficient uptake of diverse monomers and oligomers into neurons and microglia, thereby allowing trafficking to lysosomes for proteolytic detoxification.

Disclosures: U.S. Ikonne: None. **J. Hwang:** None. **B.A. Bahr:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.07/G56

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIA T32AG023477

PVAMC Merit Review Funds

Title: Copper lowering therapy for Alzheimer's disease

Authors: *K. VOSS, C. HARRIS, J. QUINN;
Neurol., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Background: Work from our lab and others has shown that high levels of copper in the brain of AD mouse models result in defects in learning and memory, increased aggregation of amyloid beta. Further, increased copper levels in AD mouse models can affect the hyperphosphorylation of tau protein.
Methods: Our lab is interested in determining if intake of oral zinc acetate, as a copper lowering therapy, is a viable strategy to reduce the effects seen in copper loaded AD mouse models. The use of zinc acetate is an FDA approved therapy for Wilson's disease, a genetic disorder that leads to increased copper accumulation in multiple organs including the brain. We tested this treatment in multiple AD mouse models, namely the Tg2576, 3xTg and hTau lines, which over express APPswe, APP/PS1/P301L tau, and wild-type human tau respectively.
Results: When oral zinc acetate is given to the mice, brain copper levels are reduced in all lines, indicating the treatment is not genotype specific. Our results also showed improvement in behavior and reduced amyloid beta load if treatment was begun early, but no effects if treatment was begun late. These data recapitulate results from our lab where copper lowering treatment

with tetrathiomolybdate improved AD like symptoms in mice if used as a preventative, but not as a treatment. We also saw modest effects on tau phosphorylation and processing in our 3xTg mice, and changes to plaque load as well. Zinc acetate had no effect on hTau mouse behavior, however, we found treatment caused a decrease in the amount of AT8 and AT180 positive tau in males, indicating a potential amyloid beta independent effect of copper loading on tau. Conclusions: These data suggest that reduction of brain copper may be a viable strategy for halting the progression of AD, through the reduction of phosphorylated tau and amyloid beta levels. If begun early enough, the treatment could have beneficial effects on the progression of the disease.

Disclosures: K. Voss: None. **C. Harris:** None. **J. Quinn:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.08/G57

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Pharmacological inhibition of BACE1 interferes with synaptic plasticity

Authors: *S. FILSER[1], C. K. E. JUNG[1], S. V. OVSEPIAN[1], A. B. ELVANG[2], C. VOLBRACHT[2], J. HERMS[1];
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Abstract: Alzheimer disease is the most common cause of dementia in the elderly with currently no therapies or proven prevention methods available. Intervening with the proteolytic generation of the amyloid-β peptide, which is believed to be the trigger of the disease, is therefore a highly aspired strategy. We analysed the effects of prolonged oral treatment of adult mice with two novel, brain-penetrant inhibitors of BACE1 - a key enzyme in the generation of amyloid-β. Both inhibitors efficiently decreased amyloid-β 1-40 levels in the cortex and plasma but caused a precarious synaptic side effect. Utilizing in vivo two-photon microscopy, we discovered a dramatic reduction in the formation of new dendritic spines in the somatosensory cortex during the inhibition of BACE1 activity; an effect which rapidly reversed after treatment was stopped. Ex vivo patch clamp analysis in pyramidal neurons of the somatosensory cortex revealed a strong reduction in the frequency of spike-driven and miniature spontaneous EPSCs. Likewise, evoked field potentials in the hippocampal CA1 area exhibited a significant reduction in the strength and activity dependent long-term synaptic plasticity of these glutamatergic inputs. Overall, while our data demonstrate the essential role of BACE1 in synaptic plasticity in various

brain areas, they also raise concerns about potential side effects caused by the therapeutic inhibition of BACE1 for Alzheimer disease.

Disclosures: S. Filser: None. **C.K.E. Jung:** None. **S.V. Ovsepian:** None. **A.B. Elvang:** None. **C. Volbracht:** None. **J. Herms:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.09/G58

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Ministry for Health and Welfare, Republic of Korea (A092042)

Ministry of Education, Republic of Korea (NRF-2012R1A1A2006801)

Asan Institute for Life Sciences, Asan Medical Center, Republic of Korea (2013-396)

Title: Anti-amyloid pathogenic activity of a metal and Aβ-interacting molecule

Authors: *J.-Y. LEE[1], S. OH[2], C. BYUN[2], M. LIM[3];
[1]Asan Med. Ctr., Asan Inst. For Life Sci., Seoul, Korea, Republic of; [2]Asan Inst. for Life Sci., Seoul, Korea, Republic of; [3]Univ. of Michigan, Ann Arbor, MI

Abstract: Because metals are involved in amyloid pathogenesis by facilitating amyloid-β (Aβ) aggregation or deposition, metal-chelator or metal-Aβ interaction blocker is considered therapeutic candidate for Alzheimer's disease (AD). A small molecule L2-b [N^1,N^1-dimethyl-N^4-(pyridin-2-ylmethyl)benzene-1,4-diamine], which interacts with both metal ions and Aβ species, can dissolve metal-induced Aβ aggregation and attenuate neurotoxicity in vitro condition (Choi et al., 2010). In this animal study using a human amyloid precursor protein (hAPP)-overexpressing 5×FAD mice, we evaluated the activity of L2-b to arrest amyloid pathology. L2-b was administered into the peritoneum of the mice every day for 21 days. Histochemical analysis using Congo-red staining or APP/Aβ-specific antibodies showed that the treatments led to the reduced load of amyloid plaques in the brain compared to vehicle-treatment. Enzyme linked immunosorbent assays (ELISAs) revealed the significant reductions in soluble and insoluble Aβ40/42 contents of the brain. Currently, we are evaluating the beneficial activity of L2-b treatments to improve the behavior or cognitive activity in 5×FAD mice.

Disclosures: J. Lee: None. **S. Oh:** None. **C. Byun:** None. **M. Lim:** None.

Poster

523. Alzheimer's Disease: Interventions

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.10/G59

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Methylene blue reverses behavioral impairment and ameliorates cerebral amyloidosis in PSAPP mice

Authors: *T. MORI[1], N. KOYAMA[1], T. SEGAWA[2], N. KINOSHITA[2], H. HOU[3], J. TAN[3], T. TOWN[4];
[1]Dept. of Biomed. Sci., Saitama Med. Ctr./Univ., Kawagoe, Japan; [2]IBL Co., Ltd., Fujioka, Japan; [3]Dept. of Psychiatry and Behavioral Neurosciences, Univ. of South Florida, Rashid Lab. for Developmental Neurobiol., Silver Child Develop. Ctr., Tampa, FL; [4]Dept. of Physiol. & Biophysics, Keck Sch. of Med. of the Univ. of Southern California, Zilkha Neurogenetic Inst., Los Angeles, CA

Abstract: Alzheimer's disease (AD) is the most common dementia and is a growing worldwide public health concern. AD neuropathological hallmarks include extracellular deposits of amyloid-β (Aβ) peptides, intracellular neurofibrillary tangles, neuronal and synaptic degeneration and loss, and neuroinflammation. Unfortunately, increasing numbers of AD pharmacotherapeutic agents have been abandoned worldwide due to toxicity issues or poor efficacy in pre-clinical rodent models and in the clinic. In this study, we tested whether the experimental agent methylene blue (MB), used for treatment of methemoglobinemia, might have anti amyloidogenic properties *in vivo*. We orally administered MB to the transgenic PSAPP mouse model of cerebral amyloidosis and evaluated cognitive function and cerebral amyloidosis. Beginning at 15 months of age, animals were gavaged with MB (3 mg/kg) or vehicle once daily for 3 months. MB treatment significantly prevented transgene-associated behavioral impairment including hyperactivity, decreased object recognition, and defective spatial reference memory, but did not alter non-transgenic mouse behavior. Moreover, brain parenchymal β-amyloid deposits and abundance of various Aβ species including oligomers were mitigated in MB-treated PSAPP mice. These effects occurred with a shift toward non-amyloidogenic amyloid precursor protein (APP) proteolysis. Specifically, we observed decreased cleavage of the β-carboxyl-terminal APP fragment and reduced β-site APP cleaving enzyme 1 protein expression and activity. These results raise the possibility that oral MB treatment may be a promising prophylaxis for AD-related cerebral amyloidosis by endorsing the anti-amyloidogenic pathway.

Disclosures: T. Mori: None. **N. Koyama:** None. **T. Segawa:** None. **N. Kinoshita:** None. **H. Hou:** None. **J. Tan:** None. **T. Town:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.11/G60

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: National Natural Sciences Foundation of China 30973511

Title: L-3-n-butylphthalide improves cognitive impairment in a transgenic ad mouse model

Authors: *Y. PENG, Y. HU, S. XU, J. LI, L. WANG, X. WANG;
Inst. of Materia Medica, Beijing, China

Abstract: Alzheimer’s disease (AD) is an age-related and irreversibly progressive neurodegenerative disorder that occurs gradually and results in memory, behavior and personality changes. L-3-n-butylphthalide (L-NBP), an extract from seeds of *Apium graveolens Linn* (Chinese celery), has been demonstrated to have neuroprotective effects on ischemic, vascular dementia and amyloid-ß (Aß)-infused animal models by inhibiting oxidative injury, neuronal apoptosis and glial activation, regulating APP processing and reducing Aß generation. In the current study, we examine the effect of L-NBP on learning and memory in amyloid precursor protein (APP) and presenilin 1 (PS1) double-transgenic AD mouse model (APP/PS1) and the mechanisms of L-NBP in reducing Aß accumulation and tau phosphorylation. Twelve-month old APP/PS1 mice were given 15 mg/kg L-NBP by oral gavage for 3 months. L-NBP treatment significantly improved the spatial learning and memory deficits compared to the vehicle-treated APP/PS1 mice, whereas L-NBP treatment had no effect on cerebral Aß plaque deposition and Aß levels in brain homogenates. However, we found a L-NBP-induced reduction of tau hyperphosphorylation at Ser199, Thr205, Ser396 and Ser404 sites in APP/PS1 mice. At the meantime, the expressions of cyclin-dependent kinase (CDK-5) and glycogen synthase kinase 3ß (GSK-3ß), the most important kinases involved in tau phosphorylation, were markedly decreased by L-NBP treatment. The effects of L-NBP on decreasing tau phosphorylation and kinases activations were further confirmed in neuroblastoma SK-N-SH cells over-expressing WT human APP_{695} (SK-N-SH APPwt). L-NBP shows promising candidate of multi-target neuronal protective agent for the treatment of Alzheimer’s disease.

Disclosures: Y. Peng: None. **Y. Hu:** None. **S. Xu:** None. **J. Li:** None. **L. Wang:** None. **X. Wang:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.12/H1

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Subchronic donepezil prevents amyloid-beta-induced memory disruption in the rat

Authors: *S. WAGNER, E. POIRAUD, N. KADOUCI, E. ANDRIAMBELOSON; NEUROFIT, ILLKIRCH, France

Abstract: Clinical studies have shown that donepezil potentially impacts the pathophysiology of Alzheimers's disease. In the present study, the neuroprotective effect of donepezil against amyloid-beta-induced memory dysfunction was investigated in the rat. Amyloid-beta (15 µg/rat) was stereotactically injected intracerebroventricular (i.c.v.) in male Wistar rats. After 2 weeks, memory performance was assessed using the passive avoidance test.
Subchronic donepezil (0.3 mg/kg/d) significantly reduced the number of trials to acquisition and prevented passive avoidance disruption in i.c.v. amyloid-beta injected rats. In contrast, acute donepezil (0.3 mg/kg) administered 30min prior the passive avoidance test did not modify the number of trials to acquisition and did not prevent passive avoidance disruption of i.c.v. amyloid-beta injected rats. It is noteworthy that acute donepezil significantly improved the memory performance of naïve rats as assessed in the passive avoidance test. The above findings suggest that subchronic donepezil interfered with the process of amyloid-beta -induced memory disruption in the rat. This beneficial effect of donepezil appeared unrelated to its acute cognitive enhancing property.

Disclosures: S. Wagner: None. **E. Poiraud:** None. **E. Andriambeloson:** None. **N. Kadouci:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.13/H2

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Grant Agency of the Czech Republic, project P303/12/0611

Title: Therapeutic effect of cholinesterase inhibitors rivastigmine, donepezil and tacrine on cognitive deficit induced by 3-quinuclidinyl benzilate in rats performing passive avoidance test

Authors: *J. MISIK[1], K. MUSILEK[2], K. KUCA[2], J. KASSA[2], O. SOUKUP[3];
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Abstract: The central cholinergic system is significantly linked with tasks of learning and memory. A shortage of cholinergic transmission is connected with cognitive alterations, a typical sign accompanying neurodegenerative diseases. Cholinesterase inhibitors, considered for the palliative treatment of patients suffering from Alzheimer's disease, were tested for their efficacy in the treatment of experimentally induced cholinergic depletion. The aim of this study was to compare the therapeutic effect of inhibitors tacrine, rivastigmine and donepezil on cognitive impairment induced by cholinergic antagonist 3-quinuclidinyl benzilate (QNB) in rats. Male Wistar rats were divided into groups per 8 and subjected to the step-through passive avoidance task with one training session and a test session after 24 hours. Rats were administered with 2 $mg.kg^{-1}$ QNB intraperitoneally before training session (30 min), followed by therapeutic dose of tacrine (3.0 and 6.0 $mg.kg^{-1}$) rivastigmine (0.6 and 1.2 $mg.kg^{-1}$) or donepezil (0.75 and 2.65 $mg.kg^{-1}$) in 15 minutes. The therapeutic doses were responsible for inhibition of brain cholinesterase in the range of 10 - 40 %. The retention entrance latency of rats treated with inhibitory drugs were compared to that of non-treated, which were administered with QNB followed by saline instead of inhibitory drug and blank controls, treated with saline instead of both, QNB and a drug. QNB significantly impaired the behavioral response of rats - QNB-administered rats showed shorter test entrance latency than control rats (Kruskal-Wallis test, $p< 0.001$). When treated, the retention latency was significantly improved in both rivastigmine-treated groups ($p < 0.05$) corresponding to cholinesterase inhibition 10 and 40%, respectively. There was no curative effect in both donepezil-treated groups and tacrine-treated groups at tested doses. Moreover, 2 out of 8 tacrine-treated rats showed signs of cholinergic over-stimulation at the higher dose of tacrine (6 $mg.kg^{-1}$). In summary, rivastigmine at the dose of 0.6 $mg.kg^{-1}$ was the most effective in reversion of QNB-induced behavioural deficit.

Disclosures: J. Misik: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Grant Agency of the Czech Republic, project P303/12/0611.. **K. Musilek:** None. **K. Kuca:** None. **J. Kassa:** None. **O. Soukup:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.14/H3

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: ANR (Adontage, Adoratau)

France Alzheimer

LECMA

LabEx DISTALZ

FUI Medialz

Inserm

UL2

Title: Effects of caffeine intake and adenosine A2A receptor deletion in a transgenic model of Alzheimer's Disease-like Tau pathology

Authors: *D. BLUM[1], C. LAURENT[1], S. BURNOUF[1], B. FERRY[2], E. MARCINIAK[1], M. DERISBOURG[1], S. EDDARKAOUI[1], S. PARROT[2], D. DEMEYER[1], C. LEDENT[3], C. MÜLLER[4], N. SERGEANT[4], M. HAMDANE[4], S. HUMEZ[1], L. LOPES[5], L. BUEE[1]; [1]Inserm U837, Alzheimer & Tauopathies, Lille, France; [2]Inserm U1028, Ctr. de Recherche en Neurosciences, Lyon, France; [3]IRIBHM, Brussels, Belgium; [4]Pharmaceut. Institute, Univ. of Bonn, Bonn, Germany; [5]Inst. de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisbon, Portugal

Abstract: Alzheimer’s disease (AD) is characterized by extracellular amyloid deposits and intraneuronal neurofibrillary tangles, made of aggregated hyper- and abnormally phosphorylated Tau proteins. The latter, referred to as “Tau pathology”, contribute to synaptic impairments leading to memory deficits in AD patients. Previous epidemiological studies revealed that habitual caffeine consumption reduces the risk to develop AD. Experimental studies suggested that chronic caffeine treatment mitigates spatial memory impairments and Aβ load in APP transgenic mice. Further, caffeine was also shown to reduce Aβ neurotoxicity, through A2A-receptor blockade. However, effects of caffeine and A2A receptor blockade towards Tau pathology remains unknown so far. The present study was aimed at evaluating the impact of chronic caffeine intake and A2A receptors deletion on memory deficits and neuro-inflammatory processes in a transgenic mouse model (THY-Tau22) exhibiting progressive hippocampal Tau pathology.

Caffeine (0.3g/L) was chronically administered through drinking water to Tau mice and littermate controls from 2 to 12 months of age. At completion of the treatment, caffeine was readily detected in the brain and plasma of treated animals. Results showed that caffeine treatment improved spatial memory in THY-Tau22 mice. That was associated with reduced Tau phosphorylation and proteolysis. Further, caffeine treatment significantly reduced hippocampal neuro-inflammation as shown by the reduction of several pro-inflammatory markers previously found overexpressed in THY-Tau22 mice. Effects of caffeine were partly mimicked by A2A receptor deletion. Indeed, loss of A2A receptors prevented Tau-dependent spatial memory alterations, an effect associated with a normalization of the hippocampal Glutamate/GABA ratio as seen obtained using microdialysis technique. Further, A2A receptor deletion led to a significant reduction of hippocampal Tau phosphorylation. Finally, THY-Tau22 A2A KO mice exhibited reduced hippocampal neuro-inflammation. Altogether, the present data are the first reporting that caffeine intake and A2A receptor deletion exert beneficial effect in a Tau transgenic mouse model of AD. These data support the therapeutic potential of both caffeine and A2A receptor antagonists in AD.

Disclosures: D. Blum: None. **C. Laurent:** None. **S. Burnouf:** None. **B. Ferry:** None. **E. Marciniak:** None. **M. Derisbourg:** None. **S. Eddarkaoui:** None. **S. Parrot:** None. **D. Demeyer:** None. **C. Ledent:** None. **C. Müller:** None. **N. Sergeant:** None. **M. Hamdane:** None. **S. Humez:** None. **L. Lopes:** None. **L. Buee:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.15/H4

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Intravenous neprilysin reduces peripheral Aβ in mouse but does not correlate with brain amyloid burden

Authors: *A. M. SCHUMACHER[1,2], R. PACOMA[2], J. WATSON[2], W. OU[2], J. ALVES[2], D. E. MASON[2], E. C. PETERS[2], H. D. URBINA[2], G. WELZEL[2], A. ALTHAGE[2], B. LIU[2], T. TUNTLAND[2], L. H. JACOBSON[3], J. L. HARRIS[2], J. R. WALKER[2];
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Abstract: Pathological aggregation and buildup of beta-amyloid (Aβ) in the brain is a hallmark of Alzheimer’s Disease (AD). Aβ levels are regulated by production from the

amyloid precursor protein, proteolytic degradation, and peripheral clearance. In this study we investigated whether enhancing clearance of plasma Aβ via peripheral administration of the Aβ-degrading protease neprilysin (NEP) would reduce brain Aβ levels through a peripheral sink. We developed an Fc-NEP fusion protein that demonstrated *in vitro* degradation of Aβ and a 10 day plasma half-life in mouse. Intravenous delivery of this active NEP protein to WT and APP23 transgenic mice resulted in a dose-dependent reduction of plasma Aβ. However, this did not correspond to a reduction in the levels of soluble brain Aβ with treatment up to 5 weeks in WT mice or formic acid-extractable brain Aβ with 3 month treatment in aged APP23. In contrast, direct intracranial injection of the Fc-NEP resulted in an acute decrease in soluble brain Aβ. These results suggest a lack of a robust peripheral Aβ efflux sink through which brain amyloid burden can be therapeutically reduced.

Disclosures: **A.M. Schumacher:** A. Employment/Salary (full or part-time):; Genomics Institute of the Novartis Research Foundation. **R. Pacoma:** A. Employment/Salary (full or part-time):; Novartis. **J. Watson:** A. Employment/Salary (full or part-time):; Novartis. **W. Ou:** A. Employment/Salary (full or part-time):; Novartis. **J. Alves:** None. **D.E. Mason:** A. Employment/Salary (full or part-time):; Novartis. **E.C. Peters:** A. Employment/Salary (full or part-time):; Novartis. **H.D. Urbina:** A. Employment/Salary (full or part-time):; Novartis. **G. Welzel:** None. **A. Althage:** A. Employment/Salary (full or part-time):; Novartis. **B. Liu:** A. Employment/Salary (full or part-time):; Novartis. **T. Tuntland:** A. Employment/Salary (full or part-time):; Novartis. **L.H. Jacobson:** None. **J.L. Harris:** A. Employment/Salary (full or part-time):; Novartis. **J.R. Walker:** A. Employment/Salary (full or part-time):; Novartis.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.16/H5

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH-NCCAM AT002688

Title: Centella asiatica protects against the toxic effects of intracellular β-amyloid accumulation

Authors: ***N. E. GRAY**[1], J. MORRE[2], J. KELLEY[2], C. S. MAIER[2], J. F. STEVENS[2], A. SOUMYANATH[1], J. F. QUINN[1];
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Abstract: Background: The plant *Centella asiatica* has been used in Ayurvedic medicine as a nerve tonic and to enhance cognition, effects that have been confirmed experimentally in animals as well as human subjects. Previous research in our lab has shown that the water extract of *Centella asiatica* (CAW) corrects beta amyloid (Aβ)- induced cognitive deficits in a transgenic model of Alzheimer's disease and prevents Aβ toxicity *in vitro*. In this study we evaluate the effects of CAW on Aβ-induced alterations in neuroblastoma cells and identify potentially active compounds within the extract.
Methods: Using the MC65 neuroblastoma cell line, a model of intracellular Aβ toxicity, we evaluated the effects of CAW cell viability, Aβ expression, tau expression and phosphorylation and induction of the antioxidant response gene NRF2 (nuclear factor erythroid-derived factor 2). We also used high-resolution mass spectrometry (HRMS) and high performance liquid chromatography (HPLC) to identify compounds present in the CAW extract.
Results: CAW treatment reduced Aβ-induced cell death without changing Aβ accumulation in the cells. Additionally, CAW attenuated Aβ-induced increases in total tau expression and site specific phosphorylation of tau. CAW also robustly increased expression of NRF2 as well as its target genes.
HPLC analysis revealed that the triterpene compounds, asiatic acid, madecassic acid, asiaticoside and madecassoside, found in the plant and reported to have potent biological activities, were absent in CAW. Instead, using HRMS several caffeoylquinic acids and derivatives were determined to be present in CAW.
Discussion: Our data show a protective effect of CAW on Aβ-induced toxicity in MC65 cells. CAW prevented cell death, normalized tau expression and phosphorylation and induced expression of NRF2 and its target genes. While more studies are needed to confirm mechanisms, the effects on NRF2 suggests that the protective effect of CAW could be due, at least in part, to antioxidant actions. Additionally, the ability of CAW to prevent Aβ-induced cell death without altering overall Aβ accumulation suggests that its action is downstream of Aβ oligimerization, a potentially novel and clinically relevant mechanism of action.
Our chemical analysis also identified several caffeoylquinic acid compounds were present in CAW. This class of compounds has been shown to have neuroprotective effects against exogenous Aβ administration and to induce antioxidant response genes, therefore it is possible they are the active constituents in CAW. Studies are currently underway to determine the efficacy of these compounds in the MC65 model system.

Disclosures: N.E. Gray: None. **J. Morre:** None. **J. Kelley:** None. **C.S. Maier:** None. **J.F. Stevens:** None. **A. Soumyanath:** None. **J.F. Quinn:** None.

Poster

523. Alzheimer's Disease: Interventions

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.17/H6

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: The histamine H3 receptor antagonist SAR110894D prevents the development of Tau neurofibrillary tangles and improves cognitive deficits in a mouse model of tauopathy

Authors: J. STEMMELIN[1], V. BLANCHARD[1], N. SCHUSSLER[1], M. LOPEZ-GRANCHA[1], J. MENAGER[1], V. MARY[1], P. DELAY-GOYET[1], *G. A. BOHME[2], T. ROONEY[1], L. PRADIER[1], J. J. ALAM[1], S. CLAUDEL[1], P. BARNEOUD[1];
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Abstract: SAR110894D is a selective histamine H3 receptor (H3R) antagonist with sub-nanomolar affinity for human and rodent H3Rs and pro-cognitive activity in vivo[1]. Although H3R antagonists have been initially positioned as a treatment for the cognitive symptoms of Alzheimer's disease through enhancement of neurotransmitter release, recent data have highlighted their potential to decrease tau phosphorylation in animal models. In the present study we have examined the effect of SAR110894D on tau hyperphosphorylation and the formation of neurofibrillary tangles (NFTs) in a tau transgenic mouse model (THY-Tau22) which selectively overexpresses human 4-repeat tau (with double G272V/P301S mutations) in brain neurons and displays age-dependent tau pathology. SAR110894D was evaluated by using preventive (2 weeks or 6 months treatment starting at 2.5-3 months of age) treatment paradigm in Tau-22 mice. SAR110894D (0.002 and 0.02% in drinking water eq. 1-10mg/kg/d o.d.) had no effect on tau hyperphosphorylation at pSer199-202/Thr205 (AT8 epitope) and did not increase Ser9-GSK3β phosphorylation in the cortex of 3 month-old mice after 2 weeks treatment. By contrast, when 2.5 month old mice were treated with SAR110894D (0.00034%, 0.0034% and 0.034% in food, eq. 0.1-1-10mg/kg/d, o.d.) for 6 months, SAR110894D decreased both tau hyperphosphorylation at pSer396-pSer404 (AD-2, -37% @ 0.0034%) and pSer199-202/Thr205 (AT8, -47% @ 0.0034%) in the cortex and the formation of NFTs (Gallyas staining) in the cortex (-48% @ 0.0034%), hippocampus (-25% @ 0.0034%) and amygdala (-31% @ 0.034%). Ser9-GSK3β phosphorylation was not modified at any doses tested. Moreover, SAR110894D (0.00034%, 0.0034%) decreased the expression of macrophage inflammatory protein (MIP)-1α mRNA (a marker of microgliosis) in the hippocampus by 35% and 36%, respectively. In addition, SAR110894D improved episodic shape and spatial memory deficits in THY-Tau22 mice after 6 months of treatment. These results demonstrate that SAR110894D prevents tau hyperphosphorylation and the formation of NFTs after chronic, but not acute, treatment and suggest that long term SAR110894D treatment could have potential disease modifying activity in Alzheimer's disease.

[1] Griebel et al (2012) Pharmacol Biochem Behav. 102(2):203-14

Disclosures: **J. Stemmelin:** A. Employment/Salary (full or part-time):; Sanofi. **V. Blanchard:** A. Employment/Salary (full or part-time):; Sanofi. **N. Schussler:** A. Employment/Salary (full or part-time):; Sanofi. **M. Lopez-Grancha:** A. Employment/Salary (full or part-time):; Sanofi. **J. Menager:** A. Employment/Salary (full or part-time):; Sanofi. **V. Mary:** A. Employment/Salary (full or part-time):; Sanofi. **P. Delay-Goyet:** A. Employment/Salary (full or part-time):; Sanofi. **G.A. Bohme:** A. Employment/Salary (full or part-time):; Sanofi. **T. Rooney:** A. Employment/Salary (full or part-time):; Sanofi. **L. Pradier:** A. Employment/Salary (full or part-time):; Sanofi. **J.J. Alam:** A. Employment/Salary (full or part-time):; Sanofi. **S. Claudel:** A. Employment/Salary (full or part-time):; Sanofi. **P. Barneoud:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.18/H7

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Mesenchymal stem cells increase hippocampal neurogenesis and neuronal differentiation by enhancing the Wnt signaling pathway in Alzheimer’s disease model

Authors: *H. PARK;
Neurol., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: ABSTRACT
Neurogenesis in the subgranular zone (SGZ) of the hippocampal dentate gyrus may act as an endogenous repair mechanism in Alzheimer’s disease (AD) and the Wnt signaling pathway has been suggested to closely modulate neurogenesis in amyloid-β (Aβ)-related AD models. The present study investigated whether mesenchymal stem cells (MSCs) would modulate hippocampal neurogenesis via modulation of the Wnt signaling pathway in a model of AD. In Aβ-treated neural progenitor cells (NPCs), the co-culture with MSCs increased significantly the expression of GFAP, SOX2, and HuD compared to Aβ treatment alone. In addition, MSC treatment in Aβ-treated NPCs enhanced the expression of Wnt3a, β-catenin, and Ngn1 compared to Aβ treatment alone. MSC treatment in Aβ-treated animals significantly increased the number of BrdU-ir cells in the hippocampus at 2 and 4 weeks compared to Aβ treatment alone. In addition, quantitative analysis showed that the number of BrdU and HuD double-positive cells in the dentate gyrus was significantly higher in the MSC-treated group than in controls or after Aβ treatment alone. These results demonstrate that MSC administration significantly augments hippocampal neurogenesis and enhances the differentiation of NPCs into mature neurons in AD

models by augmenting the Wnt signaling pathway. The use of MSCs to modulate endogenous adult neurogenesis may have a significant impact on future strategies for AD treatment.

Disclosure.DisclosureBlock:

Poster

523. Alzheimer's Disease: Interventions

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.19/H8

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Selectivity and pharmacokinetic profile of highly oligomer-specific amyloid-beta antibodies

Authors: *S. BARGHORN[1], A. STRIEBINGER[1], S. GIAISI[1], B. BEHL[1], E. TARCSA[2], C. GRINNELL[2], H. HILLEN[1];
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Abstract: Background:
Amyloid-beta (Aβ) fibril-containing amyloid plaques in the brain are a hallmark of Alzheimer's disease (AD) but a recent paradigm shift indicates Aβ oligomers as crucial neuropathogenic agents in AD. Using an *in vitro* generated Aβ oligomer, the truncated Aβ(20-42) Globulomer (tAβ Globulomer), we generated a humanized, Aβ oligomer-specific antibody, ABT-736 for use in passive immunotherapy of AD. ABT-736 selectively detects Aβ oligomers in the brains of AD patients, APP Tg-mice and in vitro. The tAβ Globulomer itself is also an ideal antigen for active immunotherapy of AD as the elicited immune response is Aβ oligomer selective.
Here, we show the efficacy of tAβ Globulomer immunization in reducing Aβ oligomer levels in Tg2576 mice and the favorable pharmacokinetic profile of ABT-736 due to its high Aβ oligomer selectivity.
Results:
ABT-736 in cynomolgus monkeys (single dose, 5 mg/kg, IV or SC) exhibited a favorable pharmacokinetic profile, including long serum half-life of 7.5 - 9 days (SC / IV respectively) of the free antibody.
To demonstrate the specificity of ABT-736, anti-Aβ antibodies were injected 2h prior to following the plasma clearance of injected 125Iodine-Aβ1-40 in APP/Lo mice. A pan Aβ antibody prevented the rapid clearance of 125I-Aβ1-40. In contrast, ABT-736 that does not bind

to monomeric Aβ *in vivo* did not impact the elimination of the peptide, it was as efficiently cleared from blood as in the presence of an unspecific IgG antibody.
For active tAβ Globulomer immunization we used Tg2576 mice (3 month). At 12 month age without any further boosting tAβ Globulomer titers were ~ 1:200-4000. This titer proved sufficient to reduce Aβ oligomer levels in the hippocampus by 50% (p=0.01) compared to control mice.
Conclusion:
We propose that exclusively targeting Aβ oligomers as neuropathogenic agents of AD with Aβ oligomer selective antibodies like ABT-736 or an active tAβ Globulomer immunotherapy has major advantages.
The high selectivity of ABT-736 for Aβ oligomers eliminates its binding to, and hence its consumption by, other non-disease related Aβ species, such as Aβ monomers, sAPPα, Aβ fibrils. This selectivity, firstly reduces potential side effects and secondly results in a favorable PK profile of ABT-736. In summary, these characteristics provide passive ABT-736 or active tAβ Globulomer immunotherapy with a highly favorable profile compared to Aβ peptide unselective immunotherapy for AD.
Disclosure:
SB, AS, SG, BB, ET, CG and HH are employees of AbbVie and may own AbbVie stock.
This study was sponsored by AbbVie. AbbVie contributed to the study design, research, and interpretation of data, writing, reviewing and approving the publication.

Disclosures: S. Barghorn: A. Employment/Salary (full or part-time):; AbbVie GmbH & Co KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie GmbH & Co KG. **A. Striebinger:** A. Employment/Salary (full or part-time):; AbbVie. **S. Giaisi:** A. Employment/Salary (full or part-time):; AbbVie. **B. Behl:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **H. Hillen:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **E. Tarcsa:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **C. Grinnell:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie.

Poster

523. Alzheimer's Disease: Interventions

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.20/H9

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG023084

NIH Grant NS034467

Title: A lipoprotein receptor cluster IV mutant preferentially binds amyloid-β and regulates its clearance from the mouse brain

Authors: *A. P. SAGARE[1], R. D. BELL[2], A. SRIVASTAVA[3], J. D. SENGILLO[1], I. SINGH[2], Y. NISHIDA[2], N. CHOW[3], B. V. ZLOKOVIC[1];
[1]Dept. of Physiol. and Biophysics, USC, Los Angeles, CA; [2]Ctr. for Neurodegenerative and vascular Brain Disorders, Univ. of Rochester, Rochester, NY; [3]ZZ Alztech, Rochester, NY

Abstract:
Soluble low-density lipoprotein receptor-related protein-1 (sLRP1) binds ~ 70% of amyloid β-peptide (Aβ) in human plasma. In Alzheimer's disease (AD) and individuals with mild cognitive impairment converting to AD, plasma sLRP1 levels are reduced and sLRP1 is oxidized which results in diminished Aβ peripheral binding and higher levels of free Aβ in plasma. Experimental studies have shown that free circulating Aβ re-enters the brain and that sLRP1 and/or its recombinant wild-type cluster IV (WT-LRPIV) prevent Aβ from entering the brain. Treatment of Alzheimer's *APPsw*$^{+/0}$ mice with WT-LRPIV has been shown to reduce brain Aβ pathology. In addition to Aβ, LRPIV binds multiple ligands. To enhance LRPIV binding for Aβ relative to other LRP1 ligands, we generated a library of LRPIV-derived fragments and full-length LRPIV variants with glycine (G) replacing aspartic acid (D) residues 3394, 3556 and 3674 in the calcium binding sites. Compared to WT-LRPIV, a lead LRPIV-D3674G mutant had 1.6-fold and 2.7-fold higher binding affinity for Aβ40 and Aβ42 *in vitro*, respectively, and a lower binding affinity for other LRP1 ligands, e.g., apoliporotein E2, E3 and E4 (1.3-1.8-fold), tissue plasminogen activator (2.7-fold), matrix metalloproteinase-9 (4.1-fold) and Factor Xa (3.8-fold). LRPIV-D3674G cleared mouse endogenous brain Aβ40 and Aβ42 25-27% better than WT-LRPIV. A 3-month subcutaneous treatment of *APPsw*$^{+/0}$ mice with LRPIV-D3674G (40 μg/kg/day) reduced Aβ40 and Aβ42 levels in the hippocampus, cortex and cerebrospinal fluid by 60-80%, and improved cerebral blood flow responses and hippocampal function at 9 months of age. Thus, LRPIV-D3674G is an efficient new Aβ clearance therapy.

Disclosures: A.P. Sagare: None. **R.D. Bell:** None. **J.D. Sengillo:** None. **B.V. Zlokovic:** Other; BVZ is the scientific founder of Socratech LLC, a startup biotechnology company with a mission to develop new therapeutic approaches for stroke and Alzheimer disease.. **I. Singh:** None. **Y. Nishida:** None. **A. Srivastava:** None. **N. Chow:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.21/H10

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Drug Discovery Foundation

Appel Alzheimer's Disease Research Institute

Title: AAV-mediated delivery of apoE2 to reduce AD neuropathology in transgenic mice

Authors: *L. ZHAO[1], A. J. GOTTESDIENER[1], M. LI[3], C. M. GREVSTAD[1], S. M. KAMINSKY[2], M. J. CHIUCHIOLO[2], D. SONDHI[2], R. G. CRYSTAL[2], S. M. PAUL[1]; [1]Appel Alzheimer's Dis. Res. Institute, Mind and Brain Res. Inst., [2]Dept. of Genet. Med., Weill Cornell Med. Col., New York, NY; [3]Dept. of Neurol. and Hope Ctr. for Neurolog. Disorders, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: The common apolipoprotein E (*APOE*) alleles are important genetic risk factors for late-onset Alzheimer’s disease (AD). *APOE*4 carriers have a markedly increased risk for developing AD (3-15 fold for heterozygotes and homozygotes respectively) whereas *APOE*2 is protective, reducing AD risk by 50% and markedly delaying the age of onset. We have previously reported that gene delivery of apoE2 using a lentiviral vector directly into the hippocampus of mutant amyloid precursor protein (APP) transgenic mice, decreases hippocampal Aβ levels and amyloid burden. Adeno-associated virus (AAV) vectors have demonstrated the capacity for robust and persistent gene expression in the central nervous system (CNS). To further explore gene delivery of apoE2 as a therapeutic strategy for AD, we compared several AAV serotypes for apoE2 expression in the CNS and evaluated changes in amyloid pathology in a transgenic amyloid-depositing mouse model of AD. We first compared expression of the apoE2 gene in apoE knock-out (EKO) mice mediated by each of several AAV serotypes, promoters and routes of delivery. We found that AAV8, AAV9 and AAVrh.10 mediate efficient apoE2 expression in brain, and the astrocytic promoter for glia fibrillary acidic protein (GFAP) results in astrocyte-specific expression of apoE2 at physiological levels. By contrast, AAV serotype vectors with the constitutive CBA promoter result in 5-6 times higher levels of apoE2

expression in brain. We next injected AAV8-GFAP-apoE2 or AAVrh.10-CBA-apoE2 into the hippocampus of 9-month old PDAPP transgenic mice. After 8 weeks, AAVrh.10-CBA-apoE2 treatment resulted in a marked decrease in both soluble (~33% reduction. $p < 0.05$) and insoluble Aβ1-42 levels (~70% reduction. $p < 0.001$) compared to control mice. In contrast, AAV8-GFAP-apoE2 treatment resulted in only a modest reduction of soluble Aβ1-42 (17% reduction. $p < 0.05$). Our data extends our earlier findings using lentiviral-mediated gene delivery of apoE2 and suggest that supraphysiological levels of apoE2 may be essential to reduce brain amyloid burden in mice with existing substantial Aβ/amyloid deposition. Further characterization of the effects of AAV-apoE2 on brain AD pathology in transgenic mice of different ages may help to elucidate whether apoE2 gene delivery represents a potential therapeutic intervention for either preventing or treating AD.

Disclosures: L. Zhao: None. **A.J. Gottesdiener:** None. **M. Li:** None. **C.M. Grevstad:** None. **S.M. Kaminsky:** None. **M.J. Chiuchiolo:** None. **D. Sondhi:** None. **R.G. Crystal:** None. **S.M. Paul:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.22/H11

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NINDS Grant NS069375-01A1

Title: Activation of β1-adrenergic receptor rescues social memory deficit in the mouse model of Alzheimer’s disease

Authors: *P. MEMAR ARDESTANI, L. COUTELLIER, M. SHAMLOO;
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Abstract: Impaired social recognition is seen in many neurodegenerative disorders. Investigating neural basis of such defects may provide insight to the underlying mechanisms of these disorders and highlight new therapeutic approaches.
In this study, we used the Thy1-APPLond/Swe (APP) mice model of Alzheimer’s disease (AD). We show that these mice display social recognition impairments without any impairment in olfactory abilities. Due to progressive degeneration of noradrenergic system in AD and according to our new findings on the role of β1-adrenergic activation in medial amygdala (MeA) is key for social recognition, we investigated if this receptor could be targeted in experimental

AD to rescue the social recognition deficit. We have shown that treatment with xamoterol, a β1-adrenergic receptor (β1-ADR) partial agonist, rescues the social deficit observed in APP mice. We hypothesized that alterations in the pCREB signaling pathway downstream of the B1-ADR could be responsible for this effect. In conclusion, our result suggest alteration of the B1-ADR/pCREB signaling cascade is responsible for the social memory deficit seen in AD and targeting this receptor can be a potential therapeutic approach to treat social abnormalities seen in AD patients.

Disclosures: P. Memar Ardestani: None. **L. Coutellier:** None. **M. Shamloo:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.23/H12

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG021975, U0128583 (S. A. F.)

RC1 AG035878 (S. A. F.and G. M. C.)

AT006816 (G. M. C.)

P01 AG16570 (G.M.C)

VA Merit grant X001257 (S. A. F., (G. M. C.)

Alzheimer’s Association NIRG-07-59659 (Q.L.M.)

Title: A pyrazole derivative of curcumin improved brain insulin resistance and cognitive decline in a transgenic mouse model of Alzheimer’s disease

Authors: *Q.-L. MA[1,2], X. ZUO[1,2], F. YANG[1,2], Q. CHEN[1,2], S. A. FRAUTSCHY[1,3], G. M. COLE[1];
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Abstract: Growing evidence indicates that brain insulin resistance is a common and early molecular pathological feature of Alzheimer’s disease (AD) for which type II diabetes is an important risk factor. Talbot and colleagues have shown early stage AD brains have elevated

serine phosphorylation of IRS-1 corelating with its upstream activated Akt, GSK, ERK , JNK and mTOR. Our previous study reported that compared to normal control diet, feeding 3xTg-AD transgenic mice with a diet high in saturated and omega-6 fat induced significant degradation of IRS-1 that was consistently associated with basal elevation of hippocampal IRS-1 phosphorylated at serine 616 (IRS-1pS616), a key marker of insulin resistance in the periphery and AD brain. This phosphorylation was sensitive to two interventions that reduced active JNK (fish oil and curcumin). In this study, we used 3xTg-AD mice with features of basal brain insulin resistance, and treated with a pyrazole derivative of curcumin named CNB-001. This derivative has a better oral bioavailablity profile than curcumin and additional strong neuroprotective activities in models with excitotoxicity, oxidative damage and glucose starvation. We treated these mice for four-months which resulted in significant amelioration of markers related to brain insulin resistance including increases in total IRS-1 and decreases in IRS-1pS616. This was accompanied by improvement of working memory using spontaneous alteration in the Y-maze. CNB-001 also significantly lowed activated Akt, GSK-3, ERK, JNK and mammalian target of rapamycin (mTOR), which have been reported to exert feedback inhibition on insulin receptor beta and IRS-1 contributing to basal insulin resistance as measured in AD tissue and ex vivo AD brain samples. CNB-001 also reduced ptau. Our data demonstrate that dietary CNB-001 has the potential to improve insulin signaling and cognitive deficits in an AD animal model with both abeta and tau pathology and markers of basal insulin resistance resembling AD brain. Given CNB-001's other reported neuroprotective effects and the known role of the related kinase pathways in IRS-1 and tau molecular pathology as well as synaptic plasticity, CNB-001 and related compounds should be further explored as potential novel drugs for AD therapy that provide alternatives or adjuncts to simply targeting abeta.

Disclosures: Q. Ma: None. **X. Zuo:** None. **F. Yang:** None. **Q. Chen:** None. **S.A. Frautschy:** None. **G.M. cole:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.24/H13

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Thome Memorial Foundation

Alzheimer's Association Zenith Award

Alzheimer's Drug Discovery Foundation

NIH Grant R01 NS064246

NIH Grant R01 AG031311

NIH Grant F32 AG037280

NIH Grant U01 AG043415

Title: Development of novel *In vivo* molecular probes for CNS serine-threonine protein kinases that modulate synaptic dysfunction

Authors: O. ARANCIO[1], V. L. GRUM-TOKARS[2], S. M. ROY[2], J. P. SCHAVOCKY[2], B. BRADARIC[2], A. D. BACHSTETTER[3], B. XING[3], E. DIMAYUGA[3], F. SAEED[1], H. ZHANG[1], A. STANISZEWSKI[1], J. C. PELLETIER[2], G. MINASOV[2], W. F. ANDERSON[2], *L. J. VAN ELDIK[4], D. WATTERSON[2];
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Abstract: Serine-threonine protein kinases are critical to CNS function, yet there is a dearth of highly selective, CNS-active kinase inhibitors for in vivo investigations. Further, prevailing assumptions raise concerns about whether single kinase inhibitors can show in vivo efficacy for CNS pathologies, and debates over viable approaches to the development of safe and efficacious kinase inhibitors are unsettled. It is critical, therefore, that these scientific challenges be addressed in order to test hypotheses about protein kinases in neuropathology progression and the potential for in vivo modulation of their catalytic activity. Identification of molecular targets whose in vivo modulation can attenuate synaptic dysfunction would provide a foundation for future disease-modifying therapeutic development as well as insight into cellular mechanisms. Clinical and preclinical studies suggest a critical link between synaptic dysfunction in neurodegenerative disorders and the activation of p38αMAPK mediated signaling cascades. Activation in both neurons and glia also offers the unusual potential to generate enhanced responses through targeting a single kinase in two distinct cell types involved in pathology progression. However, target validation has been limited by lack of highly selective inhibitors amenable to in vivo use in the CNS. Therefore, we employed high-resolution co-crystallography and pharmacoinformatics to design and develop a novel synthetic, active site targeted, CNS-active, p38αMAPK inhibitor (MW108). Selectivity was demonstrated by large-scale kinome screens, functional GPCR agonist and antagonist analyses of off-target potential, and evaluation of cellular target engagement and mechanism of action. In vitro and in vivo assays demonstrated that MW108 ameliorates beta-amyloid induced synaptic and cognitive dysfunction. A serendipitous discovery during co-crystallographic analyses revised prevailing models about active site targeting of inhibitors, providing insights that will facilitate future kinase inhibitor design. Overall, our studies deliver highly selective in vivo probes appropriate for CNS

investigations and demonstrate that modulation of p38αMAPK activity can attenuate synaptic dysfunction.

Disclosures: O. Arancio: None. **V.L. Grum-Tokars:** None. **S.M. Roy:** None. **J.P. Schavocky:** None. **B. Bradaric:** None. **A.D. Bachstetter:** None. **B. Xing:** None. **E. Dimayuga:** None. **F. Saeed:** None. **H. Zhang:** None. **A. Staniszewski:** None. **J.C. Pelletier:** None. **G. Minasov:** None. **W.F. Anderson:** None. **L.J. Van Eldik:** None. **D. Watterson:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.25/H14

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: CIHR Grant 724181712

NIH Grant 2R01EB003268

Title: Neuronal and astrocytic differentiation following transcranial focused ultrasound

Authors: *T. SCARCELLI[1], J. F. JORDAO[2], N. ELLENS[3], M. O'REILLY[3], K. HYNYNEN[3], I. AUBERT[2];
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Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by a deterioration in cognition and the development of pathological hallmarks, including amyloid-beta peptide (Aβ) aggregation and cell loss. MRI-guided focused ultrasound (MRIgFUS) can be used to locally and reversibly increase the permeability of the blood-brain barrier (BBB) and improve therapeutic efficacy by enhancing the delivery of anti-Aβ antibodies to the brain. Studies from our laboratory have shown that MRIgFUS-delivery of anti-Aβ antibodies (BAM10) significantly reduced Aβ plaque number, size and surface area by 4 days post-treatment in a mouse model of AD (Jordão et al., 2010). We recently found that MRIgFUS treatment in the absence of BAM10 also reduced Aβ plaque size and surface area after 4 days. Here, we examined whether MRIgFUS alone and in the combination with BAM10 results in Aβ reduction after 18 days. This decline in Aβ load could contribute to the enhanced survival and differentiation of newborn cells in the hippocampus, which were also examined in this study. Stereological and ImageJ analyses were used to quantify Aβ plaque number, size and surface area in the cortex and hippocampus. Immunohistochemistry and confocal microscopy were used to quantify the population of proliferating cells that survived and differentiated into neurons and

astrocytes, through the co-localization of cell lineage markers with the proliferation marker 5-bromo-2’-deoxyuridine at 18 days post-treatment. We found that MRIgFUS in the presence of 1mg/kg BAM10 significantly reduced Aβ surface area by 18 days post-treatment, potentially stimulating neuron and astrocyte proliferation and differentiation. To examine the effects of MRIgFUS treatment alone in the absence of Aβ accumulation, treatment was applied to non-transgenic mice. MRIgFUS up-regulated cell proliferation and survival in the hippocampus, leading to enhanced neuronal and astrocytic differentiation. Overall, this study showed that MRIgFUS enhances cell proliferation, differentiation and survival in the non-transgenic mice and that the addition of the anti-Aβ therapeutic may be needed to produce similar effects in the AD-like mouse model. MRIgFUS-delivered immunotherapy could then be used to decrease Aβ load and increase neuronal and astrocytic differentiation, providing a multifaceted approach to AD treatment.

Disclosures: T. Scarcelli: None. **J.F. Jordao:** None. **N. Ellens:** None. **M. O'Reilly:** None. **K. Hynynen:** None. **I. Aubert:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.26/H15

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: 1RO1 AG02980201 NIA/NIH

Title: Neuroprotective effect of flavonoids in a triple transgenic alzheimer's disease mice model

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Abstract: Alzheimer's disease (AD) is the most common dementia in the world, which is characterized by progressive loss of cognitive functions. For this disease, palliative treatments have been proposed, which don´t prevent or reverse the progression of disease. In this study, we evaluated the neuroprotective effect of flavonoids: quercetin (Q) and Biflavonoid fraction (BF) extracted from Garcinia madruno (25 mg/kg) via ip, administered every 48 hours during 3 months in old mice (22 months) triple transgenic 3xTg-AD. The tasks of spatial learning and memory were analyzed by Morris water maze, besides neurodegeneration markers (Nissl, NeuN,

GFAP, Iba-1) and neuropathological (β-A (β-amyloid) and AT-8 (hyperphosphorylated tau), were evaluated by immunohistochemistry. Our data show that animals treated with flavonoids exhibit a significant reduction in β-A extracellular plaques, tauopathy, astrogliosis and morphological changes in microglia in regions involved in emotional and cognitive behavior as CA1 of hippocampus, the amygdala, the subiculum and in entorhinal cortex. These results were supported by biochemical findings with reduced AT-8 and reduced cleavage of APP by BACE1 (CTFβ), furthermore Q also reduced cleavage by α-secretase (CTFα). Additionally, both treatments induced a better performance in learning and spatial memory in comparison with controls (DMSO). Our data suggest that systemic administration of flavonoids have effects that reverse the main histological hallmarks and cognitive dysfunction in old mice 3xTg for Alzheimer's disease.

Disclosures: G.P. Cardona Gomez: None. **A.M. Sabogal-Guáqueta:** None. **J. Muñoz-Manco:** None. **N. Cortez-Rendón:** None. **R. Ramirez-Pineda:** None. **E. Osorio-Durango:** None.

Poster

523. **Alzheimer's Disease: Interventions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 523.27/H16

Topic: B.02. Ligand Gated Ion Channels

Support: Strategic Research Council, COGNITO

Lundbeck Foundation

Title: The Ly-6 proteins prostate stem cell antigen (PSCA) and Ly6H are increased in frontal cortex in Alzheimer’s disease

Authors: *M. M. JENSEN, J. D. MIKKELSEN, M. S. THOMSEN;
Neurobio. Res. Unit, Copenhagen, Denmark

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder, characterized by impaired cholinergic neurotransmission and altered expression and function of the most abundant subtypes of nicotinic acetylcholine receptors (nAChRs), the α7 and α4β2. These receptors are highly implicated in cognitive function and associated to the pathology of AD. However, studies on nAChR protein levels in AD have yielded inconsistent results with both down-regulation and no change having been reported. In recent years, interest in the α-bungarotoxin-like Ly-6 protein family has increased after the discovery that several members of the Ly-6 protein family bind to

and modulate the function of the nAChRs with an important impact on synaptic plasticity. Given the interaction between nAChRs and Ly-6 proteins, we here investigated whether Ly-6 proteins are altered in AD. We analyzed the protein levels of prostate stem cell antigen (PSCA), Ly6H, and Lypd6 in post-mortem tissue of medial frontal gyrus from non-demented controls (n=8) and AD (n=7) using western blotting. To further investigate a possible mechanism, we also analyzed the expression of the Ly-6 proteins in frontal cortex of transgenic Tg2576 mice over-expressing APP to see whether changes in Ly-6 protein levels are influenced by amyloid pathology.
We find that PSCA and Ly6H levels are significantly increased in AD patients (198±22%, $P<0.05$ and 214±38%, $P<0.01$, respectively), whereas Lypd6 levels are unchanged (99±10%, $P=0.93$). In Tg2576 mice no significant changes in PSCA and Lypd6 levels are observed (83±11%, $P=0.40$ and 102±4%, $P=0.66$, respectively), whereas Ly6H is decreased in the Tg2576 mice (75±7%, $P<0.05$) compared to wild-type.
Since PSCA is a negative modulator of the α7 nAChR, our results suggest that the function of α7 nAChRs may be decreased in AD. Thus, PSCA could be an alternative target in the treatment of AD, where current therapies have limited effects. It seems that increased APP expression is not driving the frontal cortex increase in PSCA and Ly6H found in patients with AD. Hence, other key features of AD pathology such as neurofibrillar tangles could be implicated.**Poster**

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.01/H17

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIHgrant PO1 AG022550

NIH grant PO1 AG027956

Title: Estrogen amelioration of Abeta induced defects in mitochondria is mediated by a signaling pathway involving Drp1,ER-beta, and AKAP

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Abstract: Perturbations in dynamic properties of mitochondria, which include fission, fusion, trafficking and turnover, can lead to synaptic dysfunction, apoptosis and necroptosis, and are implicated as playing a crucial role in neurodegenerative diseases, including Alzheimer's (AD), Parkinson (PD), and Huntington (HD). It is established that accumulated Aβ in synaptic

mitochondria causes impairment of respiratory function, excessive fragmentation, and deceleration of movement. In addition, it has been shown that mitochondrial fission protein Drp1interacts with Aβ and phosphorylated tau in Alzheimer's disease neurons. Although, we are beginning to understand Aβ-induced mitochondrial defects that lead to synaptic damage and dysfunction, the molecular mechanisms underlying these defects are still unclear. Previously, we and others have shown that estrogen receptor β (ER-β) is localized in the mitochondria. Because selective ER-β modulator (DPN) can activate PKA, we reasoned that DPN- induced nongenomic ER-β signaling in the mitochondrial membrane could rescue many of the mitochondrial defects caused by soluble Aβ oligomer-induced inhibition of PKA signaling cascades. We now report that DPN treatment attenuates low-dose soluble Aβ-oligomer induced dendritic mitochondrial fragmentation and reduced mobility. Measurement of mitochondrial function reveals that Aβ treatment reduces the respiratory reserve capacity of hippocampal neuron and DPN treatment ameliorates this inhibition. Biochemical analysis shows that Aβ treatment inhibits phosphorylation of dynamin-related protein 1 (Drp1) at its PKA site, and DPN treatment ameliorates this inhibition. Further, we show that arginine methylation site spanning ERβ domain interacts with mitochondrial resident PKA-AKAP signaling complex. Taken together these results strongly indicate that ERβ binds to AKAP. Further, DPN ligand-ERβ-PKA-AKAP interaction induced signaling phosphorylate Drp1 mediated attenuation of Aβ inhibition of dynamic properties of mitochondria. Thus, our findings that mitochondrial resident ERβ specific ligand, DPN can attenuate the mitochondrial defects caused by Aβ treatment highlight the possibility that this pathway may be a useful mitochondria-directed therapeutic target for Alzheimer's disease. (This work was supported by NIH grants P01 AG022550 and P01 AG027956.)

Disclosures: S.N. Sarkar: None. **J.W. simpkins:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.02/H18

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Pfizer Inc. (Groton, CT, U.S.A.)

Science Foundation Ireland (08/INV.1/B1949)

European Union FP7 Marie Curie OXY-SENSE IAPP program

Higher Education Authority PRTLI Cycle 4 (National Biophotonics and Imaging Platform Ireland)

VIPS Program (funded by Austrian Federal Ministry of Science and Research and City of Vienna)

Marie Curie Co-fund CEMP fellowship

Title: Dimebon/Latredipine is a potent activator of AMP-activated protein kinase (AMPK) and reduces neuronal excitability

Authors: *P. WEISOVA[1], S. PEREZ ALVAREZ[2], S. KILBRIDE[2], U. ANILKUMAR[2], B. BAUMANN[2], J. JORDÁN[3], T. BERNAS[4], H. J. HUBER[2], H. DÜSSMANN[2], J. H. M. PREHN[2]; [1]Mol. Cell Biol., Vienna Biocenter, Vienna, Austria; [2]Royal Col. of Surgeons in Ireland, Dublin, Ireland; [3]Univ. de Castilla-La Mancha, Albacete, Spain; [4]Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Dimebon/Latredipine is a small molecule compound with attributed neurocognitive enhancing activities which has recently been tested in clinical trials for the treatment of Alzheimer's and Huntington's disease. Dimebon has been suggested to be a neuroprotective agent that increases mitochondrial function, however the molecular mechanisms underlying these activities have remained elusive. We here demonstrate that Dimebon, at (sub)nanomolar concentrations (0.1 nM) activates the energy sensor, AMP-activated protein kinase (AMPK). Treatment of primary neurons with Dimebon increased intracellular ATP levels and glucose transporter 3 translocation to the plasma membrane. Dimebon also increased mitochondrial uptake of the voltage-sensitive probe TMRM. Gene silencing of AMPKα or its upstream kinases, LKB1 and CaMKKβ, inhibited this effect. However, studies using the plasma membrane potential indicator $DisBAC_2(3)$ demonstrated that the effects of Dimebon on TMRM uptake were largely mediated by plasma membrane hyperpolarization, precluding a pure 'mitochondrial' mechanism of action. In line with a stabilizing effect of Dimebon on plasma membrane potential, pre-treatment with Dimebon reduced spontaneous Ca^{2+} oscillations as well as glutamate-induced Ca^{2+} increases in primary neurons, and protected neurons against glutamate toxicity. In conclusion, our experiments demonstrate that Dimebon is a potent activator of AMPK, and suggest that one of the main pharmacological activities of Dimebon is a reduction in neuronal excitability.

Disclosures: P. Weisova: None. **S. Perez Alvarez:** None. **S. Kilbride:** None. **U. Anilkumar:** None. **B. Baumann:** None. **J. Jordán:** None. **T. Bernas:** None. **H.J. Huber:** None. **H. Düssmann:** None. **J.H.M. Prehn:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.03/H19

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: R42DK081298

American Diabetes Association (Grant 1-08- RA-139

Seahorse Bioscience

Defense Security Grant 7-05-DCSA-04,

Title: Mild inhibition of the mitochondrial pyruvate carrier is neuroprotective and potentiates metabolic flexibility

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Abstract: A growing body of data has established that not only metabolic dysfunction contributes to the development of Alzheimer's Disease (AD), but also that type 2 diabetes and AD may share an overlapping etiology. As such, many anti-diabetic pharmaceuticals, such as incretin analogs and thiazolidinediones (TZDs) are experimentally neuroprotective and are being repurposed for the treatment of AD. Previous work in our laboratory has shown that TZDs are acute, specific inhibitors of the mitochondrial pyruvate carrier [MPC, Divakaruni et al. (2013) PNAS. 110:14. 5422-7], and may have pleiotropic effects including mild inhibition of the MPC that enhances the metabolic profile in skeletal muscle myocytes. Here we show that in primary cortical neurons, mild MPC inhibition with UK5099 can stimulate glucose uptake at the plasma membrane, potentiate the oxidation of alternative substrates such as ketone bodies and amino acids, protect against glutamate-induced excitotoxic injury, and lower reactive oxygen production in isolated mitochondria. Taken together, these data demonstrate that mild inhibition of the MPC is experimentally neuroprotective. Moreover, they establish the mitochondrial pyruvate carrier as a promising therapeutic target for the treatment of AD and other neurodegenerative diseases that may be rooted in dysregulated glucose metabolism.

Disclosures: A.S. Divakaruni: None. **A.N. Murphy:** None. **A.Y. Andreyev:** None. **T.P. Ciaraldi:** None.

Poster

524. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.04/H20

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Everett Endowment Fund

CIHR

Title: Mitochondrial dysfunction is associated with impairments in synaptic plasticity and memory in the TgCRND8 and 3xTg mouse models of Alzheimer's disease

Authors: W. SNOW[1], S. R. CHOWDHURY[1], S. ALASHMALI[2], C. R. LIAO[3], K. OIKAWA[4], M. RAK[3], E. THOMSON[4], C. HIRSCHMUGL[5], E. PLATT[4], M. SUH[2], K. GOUGH[3], P. FERNYHOUGH[1,4], *B. C. ALBENSI[1,4];
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Abstract: Objective. Mitochondrial dysfunction has been implicated as a disease component of Alzheimer's disease (AD). To address whether mitochondrial function is altered with the progression of AD and changes in memory, mitochondrial bioenergetic profiles, ATP assessment, pathological evaluation, long term potentiation (LTP), and memory were evaluated in the TgCRND8 and 3xTg mouse models of AD.
Methods. Morris water maze (MWM) experiments were conducted on control vs. Tg mice. Subsequently, hippocampal tissues from age-matched control and TgCRND8 mice at ages 2, 6, and 14 mos and 3xTg mice at age 12 mos were used as a source of mitochondria. LTP was also assessed in some hippocampal slices. The XF24 analyzer from Seahorse Bioscience was used to measure the rates of oxygen consumption in freshly isolated mitochondria. Amplex Red kits were used to detect the level of H_2O_2. FTIR spectrochemical imaging was used to identify amyloid plaque formation and composition in brain slices.
Results. 3xTg and TgCRND8 strains were found to have deficits in LTP and MWM parameters. In addition, mitochondrial bioenergetics parameters (basal respiration, coupled respiration, maximal uncoupled respiration, respiratory control ratio - coupled and uncoupled, spare respiratory capacity) were higher, but not significant, in hippocampal mitochondria from TgCRND8 mice at 2 mos compared to age-matched control littermates. The mitochondrial bioenergetics parameters were not found to be altered between control and TgCRND8 mice at 6 months. With the progression of age to 14 mos, coupled respiration, maximal respiration and spare respiratory capacity were decreased by 45-55% in TgCRND8 mice compared with control hippocampal mitochondria. In the 3xTg model at 12 mos, basal respiration, uncoupled

respiration and spare respiratory capacity were significantly diminished compared to control. However, the generation of H_2O_2 derived from cortical and hippocampal mitochondria, was significantly higher in controls compared to 3xTg mice. Plaques formed after 12 mos were surrounded and infiltrated with lipid.
Conclusion. These data suggest that altered mitochondrial function and oxidative stress are linked to the progression of AD, which are associated with deficits in synaptic plasticity and memory.

Disclosures: W. Snow: None. **S.R. Chowdhury:** None. **S. Alashmali:** None. **C.R. Liao:** None. **K. Oikawa:** None. **M. Rak:** None. **E. Thomson:** None. **C. Hirschmugl:** None. **E. Platt:** None. **M. Suh:** None. **K. Gough:** None. **P. Fernyhough:** None. **B.C. Albensi:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.05/H21

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH grant numbers NS077239, AG032611 and AG020197

NIH training grant number 8T32MH096331-02

NYU technology on tau immunotherapy is licensed to and is being co-developed with H. Lundbeck A/S.

Title: Uptake of tau antibodies and paired helical filament enriched tau protein in naïve and transfected human neuroblastoma cells

Authors: *D. B. SHAMIR[1], N. ROSENQVIST[4], M. D. GREGORY[2], S. RASOOL[1], J. T. PEDERSEN[4], E. M. SIGURDSSON[3];
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Abstract: Our laboratory has pioneered targeting pathological tau proteins for clearance with active and passive immunotherapies. We have shown that this approach leads to antibody uptake into neurons, clears tau aggregates from the brain and prevents functional impairments, such as cognitive decline, in mouse tauopathy models.
Antibody-mediated clearance of tau likely involves intra- and extracellular pathways. To clarify

the mechanism of action of tau antibodies, we are using several in vitro models, including naïve and transfected (0N4R P301L tau) SHSY5Y human neuroblastoma cells. To accurately quantitate antibody uptake under various conditions, we have developed a flow cytometry protocol. With this novel approach, saturation of cellular uptake of a tagged antibody (targeting the non-phosphorylated Ser396/404 tau epitope) was observed at 40-50 μg/ml after 24 h incubation. Naïve and transfected cells had similar dose uptake curves with this antibody under normal conditions. By microscopy, the tau antibody was primarily located in the endosomal-lysosomal (E-L) system as detected by co-staining with such markers. Live cell imaging with phase and confocal microscopy allowed much greater detection of internalized antibodies than the fixed confocal procedure, suggesting that the latter (fixation with several washes) leads to an under estimation of cellular antibody uptake.
To better mimic a pathological state, the cells were incubated with paired helical filament (PHF) enriched Alzheimer's Disease (AD) brain protein preparation. Uptake of tagged PHF saturated at 1 μg/ml as assessed by flow cytometry, and it was primarily found in the E-L system as detected by microscopy. Pre-incubation of the cells with PHF (0.1-10 μg/ml) for 24 h, did not affect subsequent tau antibody uptake after 24 h in either cell line. However, co-incubation with PHF (0-10 μg/ml) for 24 h dose-dependently decreased tau antibody uptake to a similar extent in naïve and transfected cells, up to 41±0.1% and 33±0.2%, respectively. This was expected as the formation of extracellular antibody-PHF complexes is likely to lead to decreased cellular antibody uptake. Under both conditions, the tau antibody and PHF co-localized within the cells as revealed by microscopy. We are currently studying the uptake mechanisms in further detail to clarify intracellular clearance mechanisms. Overall, a better understanding of underlying cellular mechanisms for tau immunotherapy and the use of our flow cytometry protocol will facilitate development of this promising approach to treat AD and related tauopathies.

Disclosures: D.B. Shamir: None. **N. Rosenqvist:** None. **M.D. Gregory:** None. **S. Rasool:** None. **J.T. Pedersen:** None. **E.M. Sigurdsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on NYU patents on tau immunotherapy.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.06/H22

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: RFBR Grant 11-04-00890

Grant of President RF Scientific Scools-3598.2014.4

Grant of President RF MK-5534 2011.4

Title: Mitochondria-targeted plastoquinone antioxidant SkQ1 prevents amyloid-β-induced impairment of long-term potentiation in rat hippocampal slices

Authors: *V. G. SKREBITSKY[1], N. A. KAPAY[1], O. V. POPOVA[1], E. V. STELMASHOOK[1], R. V. KONDRATENKO[1], N. K. ISAEV[2], D. B. ZOROV[2], V. P. SKULACHEV[2];
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Abstract: Bath application of 200 nM amyloid-β 1-42 (Aβ) to rat hippocampal slices impairs induction of long-term potentiation (LTP) of the population spike (PS) in pyramidal layer of the CA1 field of the hippocampus. Intraperitoneal injection of mitochondria-targeted plastoquinone derivative SkQ1at very low concentrations (250 nmol/kg body weight) given 24 h before the slice preparation or 1 h treatment of hippocampal slices with 250 nM SkQ1 prevents the deleterious effect of Aβ on LTP. To elucidate which part of the molecule is responsible for this type of neuroprotective activity, the effect of the analog of SkQ1 lacking plastoquinone (C12TPP) was studied. It was found that C12TPP was much less efficient in LTP protection than SkQ1 itself. It means that plastoquinone part of SkQ1 molecule is responsible for the LTP rescue. To summarize, in vivo and in vitro injection of SkQ1 compensates for Aβ-induced oxidative damage of long-term synaptic plasticity in the hippocampus, which is considered to be the main reason of memory loss and impairment of other cognitive functions associated with Alzheimer's disease (AD). Therefore, SkQ1 may be considered as a promising candidate for the treatment of early-stage of AD.

Disclosures: V.G. Skrebitsky: None. **N.A. Kapay:** None. **O.V. Popova:** None. **E.V. Stelmashook:** None. **R.V. Kondratenko:** None. **N.K. Isaev:** None. **D.B. Zorov:** None. **V.P. Skulachev:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.07/H23

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NRF grant 2012-003338

NRF grant 2012-0009221

NRF grant 2011-0030928

Title: The effect of SIRT1 on the protein degradation in cholinergic neuron

Authors: *T. KIM, H. SEO;
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Abstract: SIRT1 is a prominent member of family of NAD^+ dependent class III histone deacetylase (HDAC) and has been emphasized the importance in the regulation of metabolism, energy homeostasis, stress tolerance, cellular survival, and organismal lifespan. In this study, we have examined the cellular effects of overexpression of SIRT1 in cholinergic neuron. SIRT1 overexpression reduced the mRNA expression of mitochondrial fission gene, dynamin-related protein 1 (Drp1) and altered the level of related calcium signaling. The overexpression of SIRT1 changed the chymotrypsin activities in ubiquitin proteasome system (UPS) and the level of microtubule-associated protein 1A/1B-light chain 3 (LC3) in autophagy. The treatment of MG132, reversible proteasome inhibitor, decreased the endogenous level of SIRT1 in cholinergic neuron. We also used various inhibitors to find the function of endogenous SIRT1 in cellular protein degradation system. These results suggest that the function of SIRT1 is closely related to the regulation of mitochondrial cycle and cellular protein degradation system in cholinergic neuron.

Disclosures: T. Kim: None. **H. Seo:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.08/H24

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH/NIGMS (2 SO6 GM08016-39)

HU College of Medicine Bridge Grant

Title: Combination of ketamine and AMPA promotes survival of cells expressing amyloid-beta and presenilin: Implication for Alzheimer’s disease

Authors: *L. AKINFIRESOYE[1], K. F. MANAYE[2], Y. TIZABI[1];
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Abstract: Alzheimer's disease (AD) is a progressive age-related neurodegenerative disease characterized by cognitive impairments and formation of plaques and tangles. Cellular models whereby expression of beta amyloid (Aβ), the major component of plaques, is exaggerated are commonly used to test the efficacy of novel neuroprotective compounds. In addition to Aβ, mutation in the protein presenilin has also been shown to contribute to Alzheimer's pathology. Recently, a cellular neuroblastoma model where both beta amyloid and mutated presenilin are expressed has become available. Since in-vitro protective effects of ketamine and AMPAkines against several neuronal toxins have been observed, we hypothesized that ketamine and/or AMPA will also protect against cellular damage or death due to high expression of Aβ or Aβ and presenilin. Wild type neuroblastoma (N2a) cell line and those transfected with APP_{695} (singly transfected) or the combination of APP_{695} and presenilin (doubly transfected), were pretreated with various concentrations of ketamine (0.01 - 1.0 mM), AMPA (1- 100 nM) or their combination (ketamine 0.1 mM and AMPA 100 nM) and the survivability of the cells was determined by MTT Assay after 72 hours. Pretreatment with neither ketamine nor AMPA provided any protection against cellular loss in all cell lines. However, the combination of ketamine and AMPA had a protective effect that was more pronounced in the doubly transfected cell line. These results suggest novel glutamatergic-based intervention (i.e., combination of an NMDA antagonist with an AMPA agonist) in AD.
Supported by NIH/NIGMS (2 SO6 GM08016-39) and HU College of Medicine Bridge Grant

Disclosures: L. Akinfiresoye: None. **K.F. Manaye:** None. **Y. Tizabi:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.09/H25

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NRF grant 2011-0030928

NRF grant 2012-003338

NRF grant 2012-0009221

Title: The effects of selective histone deacetylase inhibitors in cholinergic neurons

Authors: *H. NOH, H. SEO*;
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Abstract: Some of histone deacetylase (HDAC) inhibitors are recently reported as neuroprotectors by improving synaptic plasticity, learning and memory in diverse neurodegenerative disorders. We previously studied that valproic acid (VPA), non-specific HDAC inhibitor (HDACI) increased nerve growth
factor (NGF) expression and improved an impaired memory in Alzheimer's disease model mice (Noh and Seo, 2012). To determine the effects of selective HDACI *in vitro*, various inhibitors such as apicidin, VPA, suramin, sodium butyrate, MS-275, AGK-2, nicotinamide, tubacin, etc. were administered in cholinergic neurons in culture. We detected the altered mRNA levels of trophic factors and related trophic factor receptors after HDACI treatment. We also detected the increased levels of several nuclear receptors after HDACI treatment. These data suggest that histone acetylation plays key roles in the expression and function of trophic factors in cholinergic neurons.

Disclosures: H. Noh: None. **H. Seo*:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.10/H26

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: The project of Czech Grant Agency - P303/11/1907

The Long Term Organization Develompent Project 1011.

Title: Hybrids of AChE inhibitor and memantine derivatives as candidates for Alzheimer´s disease treatment

Authors: *O. SOUKUP[1,4], J. KORABECNY[2,4], K. MUSILEK[2,5], D. JUN[3], J. ZDAROVA KARASOVA[1], J. MISIK[2], K. KUCA[3,4];
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Abstract: Current treatment of for Alzheimer´s disease (AD) consists of acetylcholinesterase inhibitors (AChEi) and memantine, which is an amantadine derivative capable of antagonizing

excitotoxicity mediated by NMDA receptors. The first introduced AChEi was tacrine, however, due to its severe side-effects (hepatotoxicity and cholinergic effects upon the gastrointestinal tract) resulted in its withdrawn from the market. On the other hand, recent development of tacrine related agents revealed that 7-methoxytacrine (7-MEOTA) as less toxic derivative with similar pharmacological profile. In the search for new drug candidates, we synthesized and biologically assessed (Ellman´s method) new 7-MEOTA and amantadine hybrids combining two different approaches, AChEi and NMDA antagonist, in a single molecule. The biological in vitro evaluation revealed that some of these molecules were good inhibitors of AChE, sometimes overcoming the efficacy of tacrine. Structure-activity study revealed that affinity towards AChE was independent on whether urea or thiourea linker was used and the best activity exerted compounds with 5 carbon linker between both pharmacophores. The most promising were also evaluated for their toxicological effects anf for pharmacokinetic properties. We believem, that some of them could be qualified as potential novel anti-AD drug candidate if antiNMDA efficacy will be confirmed as well.

Disclosures: O. Soukup: None. **J. Korabecny:** None. **K. Musilek:** None. **D. Jun:** None. **J. Zdarova Karasova:** None. **J. Misik:** None. **K. Kuca:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.11/H27

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH grant AG14930

Title: Mild impairment of the citric acid cycle in neurodegenerative diseases promotes mitophagy/autophagy

Authors: *K. BANERJEE[1], T. DENTON[2], G. E. GIBSON[1];
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Abstract: Introduction: Decreased mitochondrial function, as reflected by reduced brain glucose utilization, is an invariant feature of Alzheimer’s disease (AD). Indeed, diminished mitochondrial function is a common feature of many age-related neurodegenerative diseases. The alpha-ketoglutarate dehydrogenase complex (KGDHC), a key enzyme complex in mitochondrial energy production, is diminished in AD and many other age-related neurodegenerative disorders. If diminished mitochondrial function is either an initiating event or

a critical step in a cascade of events, then the mitochondrial deficit should be linked to the abnormal accumulation of protein aggregates as well as to oxidative and mitophagic/autophagic stress, which are hallmarks of these disorders.
Objective: The goal of this study was to determine if inhibiting just one key mitochondrial enzyme, KGDHC, would initiate these processes.
Methods: Migration of cytosolic proteasomal proteins to the mitochondria initiates mitophagy (mitochondrial breakdown). Mitochondria and cytosol from SHSY5Y cells (control and treated) were isolated by differential centrifugation. Immunoblotting with antibodies to parkin and microtubule-associated protein light chain 3 (LC3) were performed to detect migration of several proteins from cytosol to different subcellular fractions. An increased LC3II/LC3I ratio in the mitochondrial fraction is an indicator of mitophagy. Confocal imaging was performed to confirm autophagy using green "autophagy detecting dye" that binds with autophagosomes. Cell-death was measured by trypan blue exclusion method and tetramethylrhodamine (TMRM) was used for detecting mitochondrial membrane potential.
Result: Carboxyethyl succinyl phosphonate (CESP) is a specific inhibitor of KGDHC that has been shown to penetrate into cells. CESP (100 µM, 5 h) diminished the mitochondrial membrane potential by about 40%. Under these conditions, cytosolic parkin declined by 25% while mitochondrial parkin increased by 20%. The LC3II/LC3I ratio increased in the mitochondrial fraction suggesting CESP induced mitophagy. Confocal imaging using the “autophagy detecting dye” confirmed the presence of autophagy in mitochondrial fraction after CESP treatment. This dye showed that other areas of the cell were also involved in autophagy. CESP treatment increased cell death by about 15%.
Conclusion & Significance: The results suggest that proteasomal activators and/or activation of KGDHC may protect against toxicity due to altered protein translocation. An understanding of the link between mitochondria and proteasomal activity may promote the development of new therapeutic strategies.

Disclosures: K. Banerjee: None. **T. Denton:** None. **G.E. Gibson:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.12/H28

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: T32GM008805-10

Thome Memorial Foundation

Title: Rexinoids enhance LXR target gene expression in primary microglia and astrocytes

Authors: *M. LAKNER[1], C. E. WAGNER[2], P. E. CRAMER[1], G. E. LANDRETH[1];
[1]Neurosci., Case Western Reserve Univ., Cleveland, OH; [2]Sch. of Mathematical and Natural Sci., Arizona State Univ., Glendale, AZ

Abstract: Alzheimer's disease (AD) is characterized by the accumulation of amyloid beta within the brain and progressive neurodegeneration resulting from imbalance in production and clearance of soluble Aβ (sAβ) peptides. The clearance of sAβ from the brain is reliant upon apolipoprotein E (ApoE) and its lipidation status. Elevation of the lipidated forms of ApoE is associated with reduced sAβ levels, plaque burden and enhanced cognition. The expression of ApoE and its lipid transport genes ATP binding cassette A1 (ABCA1), and ABCG1 are coordinately regulated by the nuclear receptors, retinoid-X-receptor (RXR): liver-X-receptor (LXR) and RXR: peroxisome proliferator activated receptor γ (PPARγ). Recently, Cramer et al (2012) showed that treatment with the FDA approved RXR agonist Bexarotene rapidly cleared amyloid and improved cognition in murine models of the disease. We have tested RXR agonists (rexinoids) in primary glia cell cultures for their ability to induce the expression of ApoE/ABCA1/ABCG1 and to proteolytically degrade Aβ. We show that treatment of primary microglia and astrocytes with synthetic RXR agonists increases the proteolytic degradation of sAβ and this effect correlates with expression of ABCA1 and ApoE. These data confirm the hypothesis that agonism of RXRs can sufficiently promote the clearance of soluble amyloid beta and provide insight into the efficacy of the Bexarotene pharmacophore.

Disclosures: M. Lakner: None. **P.E. Cramer:** None. **G.E. Landreth:** None. **C.E. Wagner:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.13/H29

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Cyclopamine modulates APP metabolism and decreases Aβ generation

Authors: *A. G. VOROBYEVA, S. MILLER, R. LEE, P. KHANDELWAL, G. DISTEFANO, A. GANGEMI, D. MARENDA, A. SAUNDERS;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease leading to memory loss. Particularly important is the synaptic loss observed in the hippocampus, the center of learning and memory. Numerous lines of evidence suggest that Aβ, a neurotoxic peptide, initiates a cascade that ultimately results in synaptic dysfunction and eventually neuronal death. Aβ is generated from Amyloid Precursor Protein (APP) by the proteolytic process of β- and γ-secretases and alterations to this processing can result in AD. Using an *in vitro* model we observed an increase in APP-CTFs and a decrease in Aβ and AICD upon cyclopamine treatment. We observed similar effects of cyclopamine on APP metabolism using a transgeneic Drosophila AD model. These results suggest cyclopamine inhibits γ-secretase and leads to decreased APP proteolytic cleavage and Aβ generation. Taken together, our data strongly suggests cyclopamine is a novel regulator of APP metabolism and may rescue the effects of neurotoxic peptide Aβ observed in other AD models.

Disclosures: A.G. Vorobyeva: None. **S. Miller:** None. **R. Lee:** None. **P. Khandelwal:** None. **G. DiStefano:** None. **A. Gangemi:** None. **D. Marenda:** None. **A. Saunders:** None.

Poster

524. **Alzheimer's Disease: In Vitro Therapeutics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 524.14/H30

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01ES014826)

Title: Diet-enriched in palmitate triggers Alzheimer's like pathology

Authors: *O. GHRIBI, S. RAZA;
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Abstract: Several studies suggest that dietary regimens rich in fat and carbohydrates can increase the risk for atherosclerosis, type 2-diabetes, and metabolic syndrome, and may thus increase the risk for Alzheimer’s disease (AD). It is particularly relevant that dietary supplements containing key nutrients may improve cognitive functioning in demented patients. Thus, depending on their components, diets can promote AD or can represent a therapeutic avenue for this disease. It is therefore important to continue exploring the potential triggering or protective roles of diets in the pathogenesis of AD. In the present studies, we demonstrate that the saturated free fatty acid (sFFA) palmitate can trigger AD-like pathology in human neuroblastoma cells as well as wild type mice and accelerates AD-like pathology in the 3xTg-AD mice. Incubation of human neuroblastoma cells with palmitate increased levels of BACE1 levels, soluble Aβ, NF-

κB, and the endoplasmic reticulum stress marker gadd153 (also called CHOP). Inhibiting gadd153 with siRNA or with compounds that inhibit gadd153 activation precluded palmitate-induced increase in BACE1, NF-κB and Aβ. On the other hand, palmitate feeding for 3 months induces gadd153 and NF-κB activation, increases BACE1 and Aβ42 levels, and activates astrocytes and microglia in wild type mice. Furthermore, feeding a diet rich in palmitate accelerated AD-like pathology in the 3xTg-AD compared to 3xTg-AD fed a normal diet. While 3 month-old 3xTg-AD fed a normal diet for 3 months showed increased APP expression levels only, age-matched 3xTg-AD fed a diet rich in palmitate for 3 months showed increased soluble Aβ levels, increased ER stress and gadd153 activation, and synaptic loss. Altogether, our results demonstrate that palmitate can generate pathological hallmarks in vitro and in vivo relevant to AD pathology. We further suggest that ER stress-mediated gadd153 activation plays an important role in palmitate effects. Diets rich in palmitate may increase the risk for AD in humans and inhibition of gadd153 may protect against palmitate-induced AD-like pathology.

Disclosures: O. Ghribi: None. **S. Raza:** None.**Poster**

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.01/H31

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Hearst Foundation Fellowship

NIH Grant EY013434

NIH Grant EY015537

Vision Research Core Grant EY001583

Research to Prevent Blindness

The Foundation Fighting Blindness

Title: Elevated lysosomal pH and autophagy dysfunction in human fibroblasts bearing the Alzheimer's-associated presenilin 1 A246E mutation can be ameliorated with cAMP

Authors: *E. E. COFFEY[1], J. M. BECKEL[1], A. M. LATIES[2], C. H. MITCHELL[1];
[1]Anat. and Cell Biol., [2]Ophthalmology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Alzheimer’s disease is one of a number of neurodegenerative disorders characterized by the pathological accumulation of protein, both intracellularly and extracellularly. A

particularly robust autophagic phenotype is also observed in Alzheimer's disease, a pathology that includes the build-up of partially degraded protein and cellular material in enlarged lysosomes and autophagosomes. This phenomenon is reportedly accentuated by certain mutations in the presenilin 1 (PS1) subunit of gamma-secretase. It had previously been suggested that PS1 mutation exerts this effect by perturbing lysosomal pH, leading to an overall degradative inefficiency; however, subsequent studies were unable to validate this effect. The current study aimed to further assess this reported acidification deficit and some of its cellular consequences. In human skin fibroblasts containing the A246E mutation in PS1, a slight elevation of lysosomal pH, on the order a fifth to a third of a pH unit, was indeed detected. This pH elevation was accompanied by increased levels of autophagosomal markers, such as an increased ratio of LC3B-II/-I and of p62, indicating an accumulation of autophagosomes within PS1 fibroblasts, and supporting the idea that PS1-linked pH elevation results in a backlog of intracellular degradation. Further investigation by qPCR revealed increased expression of the following genes in PS1 mutated fibroblasts as compared to wild-type fibroblasts: ATP6V1B2, a subunit of the proton pump responsible for lysosomal acidification; ATG5, associated with autophagosomal membrane elongation; BECN1, involved in autophagosome genesis; and TcfEB, a transcription factor that both links lysosomal and autophagic processes and also contributes to lysosomal acidification. These findings indicate that PS1 fibroblasts also exhibit a general disruption of the lysosomal-autophagosomal axis and could indicate a compensatory response to chronic pH elevation. Finally, the current study also identified that increased levels of intracellular cAMP - previously shown to restore acidic pH to pathologically or pharmacologically disrupted lysosomes - are also able to restore pH in PS1 fibroblasts, and that this pH restoration is mirrored by a reduction in LC3B-II/-I ratio, indicating that pH restoration also improved clearance of material through the degradative system. Overall, these data suggest that the PS1 mutation not only contributes to autophagic pathology in Alzheimer's disease through elevated lysosomal pH, but that this pathology can be ameliorated by lysosomal re-acidification - perhaps indicating an untapped line of future therapeutic potential.

Disclosures: E.E. Coffey: None. **J.M. Beckel:** None. **A.M. Laties:** None. **C.H. Mitchell:** None.

Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.02/H32

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: γ-Secretase inhibitor LY450139 does not impair cognitive function in wild type mice

Authors: *N. DEVIDZE[1], S. SANKARANARAYANAN[1], B. SNYDER[1], D. BRYCE[1], A. LIN[1], C. POLSON[1], S. KEENAN[1], R. OLSON[2], J. TOYN[1], C. CONWAY[1], C. F. ALBRIGHT[1], J. MEREDITH[1], M. AHLIJANIAN[1];
[1]Neurosci. Biol., [2]Neurosci. Chem., Bristol-Myers Squibb, Wallingford, CT

Abstract: One of the major hallmarks of Alzheimer's disease (AD) is the accumulation of senile plaques composed of Amyloid-β (Aβ) deposits. The sequential cleavage of Amyloid precursor protein (APP) by β-secretase (resulting in a carboxy-terminal fragment of APP, β-CTF) and the γ-secretase enzyme complex, results in the generation of multiple Aβ peptides. Trials of γ-secretase inhibitors (GSI) in AD subjects achieved limited effects on Aβ levels, and did not improve cognitive function. In order to explore the impact of GSI on cognition, we investigated the effect of acute, or sub chronic administration of LY450139 (semagacestat) in female and male wild type (WT) mice on cognitive endpoints and brain Aβ and β-CTF levels. Mice were tested in 2 behavioral paradigms; the Y-Maze spontaneous alternation test (working memory) and the Two-Trial Y-maze test (spatial learning and memory). Mice were administered LY450139 (60 to 100 mg/kg) or vehicle PO for 1 or 8 days with behavioral testing occurring 3 hours after the final dose. Immediately after behavioral testing, brains and plasma were collected for biochemical analysis and to determine drug exposure. LY450139 treatment led to a dose dependent reduction in brain Aβ40 and Aβ42 and a corresponding increase in β-CTF. There were no changes in the cognitive measures at any dose of semagacestat, including those doses that inhibited Aβ40 and 42 and increased β-CTF. These results contrast with previous reports of cognitive decrements in WT mice given LY450139, and highlight the need for further investigation to understand the effects of γ-secretase inhibition on cognitive function.

Disclosures: N. Devidze: None. **S. Sankaranarayanan:** None. **B. Snyder:** None. **D. Bryce:** None. **A. Lin:** None. **C. Polson:** None. **S. Keenan:** None. **R. Olson:** None. **J. Toyn:** None. **C. Conway:** None. **C.F. Albright:** None. **J. Meredith:** None. **M. Ahlijanian:** None.

Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.03/H33

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: R01-AG034606

Title: Investigating the effects of β-secretase inhibitor, GRL-8234, on age-dependent synaptic deficits in APPswe;PS1deltaE9 mice

Authors: *A. L. MEGILL[1], P. C. WONG[2], A. KIRKWOOD[1], H.-S. HOE[3], H.-K. LEE[1]; [2]Pathology, [1]Johns Hopkins Univ., Baltimore, MD; [3]Neurosci., Georgetown Univ., Washington, DC

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder, causing loss of synaptic contacts and cognitive decline. Current theories implicate the production of amyloid beta (Aβ) as a key molecular event that ultimately leads to neuronal degeneration and the clinical pathology seen in AD (Hardy and Selkoe, 2002). Aβ is produced by sequential proteolytic cleavage of amyloid precursor protein (APP) by two endoproteolytic enzymes, β- and γ-secretase. Therefore, inhibiting the activity of these enzymes has surfaced as one of the major disease-modifying approaches for AD (Citron, 2004).
Equally important in developing effective therapies for disease intervention is to understand how Aβ production alters normal synaptic function and what types of synaptic functions are differentially affected by Aβ (Wang and Megill et al., 2012). Increasing evidence suggests the cognitive syndromes found in AD patients are preceded by changes in synaptic efficacy (Shankar and Walsh, 2009; Selkoe, 2002). Therefore, examining whether different strategies that target APP-processing enzymes rescue synaptic dysfunctions associated with AD is important. We found that at the Schaffer collateral inputs to CA1, APPswe;PS1deltaE9 mice show age-dependent alterations in synaptic plasticity. In particular, long-term potentiation (LTP) induced with a four-train theta burst (4xTBS) protocol was decrease in adult (6 month old) mice while long-tem depression (LTD) induced with a paired pulse 1Hz (pp-1Hz) protocol was increased. In order to try and rescue these synaptic deficits, APPswe;PS1deltaE9 mice were treated with the β-secretase inhibitor, GRL-8234. GRL-8234 has been show to rescue age related cognitive decline and decrease Aβ production in Tg2576 mice (Chang et al., 2011). While GRL-8234 was able to decrease Aβ production in the hippocampus of APPswe;PS1deltaE9 mice, it did not significantly improve LTP expression. Furthermore, GRL-8234 reduced LTP expression in wildtype mice, comparable to levels seen in APPswe;PS1deltaE9 mice. On the other hand, GRL-8234 did not negatively affect LTD in wildtype mice and we are currently testing its effects on LTD in APPswe;PS1deltaE9 mice.
Support: R01-AG034606 to AK.

Disclosures: A.L. Megill: None. **P.C. Wong:** None. **A. Kirkwood:** None. **H. Hoe:** None. **H. Lee:** None.

Poster

525. Beta and Gamma Secretase, BACE, and Presenilin

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.04/H34

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: R01GM079688

R01GM089866

CBET 0941055

Title: Palmitate induces transcriptional regulation of BACE1 and presenilin by STAT3 in neurons mediated by astrocytes

Authors: *L. LIU, R. MARTIN, C. CHAN;
Michigan State Univ., East Lansing, MI

Abstract: Deregulation of calcium has been implicated in neurodegenerative diseases, including Alzheimer's disease (AD). Previously, we showed that saturated free-fatty acid, palmitate, causes AD-like changes in primary cortical neurons mediated by astrocytes. However, the molecular mechanisms by which conditioned media from astrocytes cultured in palmitate induces AD-like changes in neurons are unknown. This study demonstrates that this condition media from astrocytes elevates calcium level in the neurons, which subsequently increases calpain activity, a calcium-dependent protease, leading to enhance p25/Cdk5 activity and phosphorylation and activation of the STAT3 (signal transducer and activator of transcription) transcription factor. Inhibiting calpain or Cdk5 significantly reduces the upregulation in nuclear level of pSTAT3, which we found to transcriptionally regulate both BACE1 and presenilin-1, the latter is a catalytic subunit of γ-secretase. Decreasing pSTAT3 levels reduced the mRNA levels of both BACE1 and presenilin-1 to near control levels. These data demonstrate a signal pathway leading to the activation of STAT3, and the generation of the amyloid peptide. Thus, our results suggest that STAT3 is an important potential therapeutic target of AD pathogenesis.

Disclosures: L. Liu: None. **R. Martin:** None. **C. Chan:** None.

Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.05/H35

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Blockage of the cholesterol biosynthesis reduces γ -secretase activity and Aβ generation

Authors: *Y. KIM;
Dept. of Biomed. Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Amyloid beta (Aβ) is one of major causative molecules for Alzheimer's disease (AD) pathogenesis. It is derived from amyloid precursor protein (APP) by sequential cleavages of β- and γ-secretases. The regulation of these components has been postulated to be an important factor for Aβ generation in the pathogenesis of AD. Molecules in γ-secretase complex and APP are present in lipid raft where cholesterol regulates integrity and flexibility of membrane protein. However, the relation between cholesterol contents and Aβ generation has been controversial. In this study, we used the AY9944 to inhibit cholesterol biosynthesis at the last step of cholesterol biosynthesis, which decreases the side effect like statin. Treatment of AY-9944 decreases the γ-secretase activity and Aβ generation by the disruption of lipid raft, in which APP and γ-secretase complex including nicastrin are colocalized. The effect of AY-9944 is measured by live cell imaging using novel FRET assay system. It suggests that the cholesterol is an important factor to modulate APP processing and Aβ generation.

Disclosures: Y. Kim: None.

Poster

525. Beta and Gamma Secretase, BACE, and Presenilin

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.06/H36

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Internal funding

Title: BACE 1 contributes to impaired neural network function and neuritic dystrophy in cortical neurons generated from patients with Alzheimer's disease

Authors: *V. DANG, J. BRIGHT, S. HUSSAIN, L. NGUYEN, E. BEATTI, Z. YANG, S. WRIGHT, U. SHOUKAT-MUNTAZ, J. DIMOS, S. OIRION, P. CONLEY, N. STAGLIONO, I. GRISWALD-PRENNER;
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Abstract: Altered neuronal network activity and degeneration of neuronal processes have been linked to memory loss and impaired cognitive function associated with Alzheimer's disease (AD). To understand how these processes develop and progress, cortical neurons (CNs) were differentiated from induced pluripotent stem cells (iPSCs) derived from healthy and AD patients (familial and sporadic). Using synaptically driven bursts of action potentials as a measure of

neural network activity, we report that AD patient-derived CNs have reduced bursting activity over time ($p < 0.03$; one-tail paired t-test), whereas parallel cultures of healthy patient-derived CNs show increased bursting activity over time ($p < 0.001$; one-tail paired t-test). Concurrent with the reduction of bursting activity, CNs that failed to burst also have dystrophic neurites and reduced miniature excitatory post-synaptic current frequency. Treatment with a BACE 1 inhibitor reduced the levels of A-Beta in the culture media and prevented the decrease in bursting activity and neuritic dystrophy in familial and sporadic AD patient-derived CNs. These results suggest that BACE 1 contributes to impaired network activity and neuritic dystrophy in AD patient-derived CNs and that iPSC-derived CNs are a relevant disease model for the study of mechanisms of neuronal network dysfunction in AD.

Disclosures: V. Dang: A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **S. Oirion:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **I. Griswald-Prenner:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **J. Bright:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **L. Nguyen:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **S. Hussain:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **S. Wright:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **E. Beatti:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **Z. Yang:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **U. Shoukat-Muntaz:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **J. Dimos:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **N. Stagliono:** A. Employment/Salary (full or part-time):; iPierian Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPierian Inc. **P. Conley:** A. Employment/Salary (full

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Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.07/I1

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: P01 AG017617

Title: Presenilin maintains lysosomal calcium homeostasis by regulating v-ATPase-mediated lysosomal acidification

Authors: J.-H. LEE[1,2], D. WOLFE[1], M. MCBRAYER[1], L. HASLETT[5], A. KUMAR[1,3], Y. SATO[1], P. MOHAN[1,2], E. COFFEY[6], C. MITCHELL[7,8], E. LLOYD-EVANS[5], *R. A. NIXON[9,1,4];
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Abstract: We previously reported that presenilin-1 (PS1) deletion in mouse blastocysts, neurons, and PS1 mutation in Alzheimer's patient fibroblasts selectively inhibits lysosomal proteolysis and autophagy by blocking lysosomal acidification (Lee et al. Cell, 2010). Here we demonstrate that the V0a1 subunit of vATPase is essential for lysosomal acidification and that its maturation, lysosomal delivery, and function are strikingly deficient in neurons and MEFs from mice lacking PS1. In highly purified lysosomes of PS1KO cells, levels of V0a1 subunit are less than 20% of wild-type (WT) levels and lysosomal proton translocation activity is reduced by approximately 50%. siRNA knockdown of either V0a1 subunit or PS1 in WT neuronal cells reproduces the complete phenotype of lysosomal abnormalities and autophagy failure seen in PS1 KO cells. Elevated lysosomal pH in PS1-deficient cells causes abnormal efflux of calcium from lysosomes and a rise in cytosolic calcium levels. Restoring lysosomal calcium to normal levels in PS1KO cells neither re-acidifies lysosomes nor reverses lysosomal proteolytic and autophagic deficits. By contrast, re-acidification of lysosomes of PS1-deficient cells using acidic nanoparticles targeted to lysosomes reverses autophagy failure and restores normal lysosomal proteolysis and calcium homeostasis. Moreover, elevating lysosomal pH by blocking vATPase activity with concanamycin A induces lysosomal calcium efflux in WT cells but does not worsen the

abnormal calcium efflux in PS1KO cells, which is already enhanced by vATPase deficiency. Collectively, our studies show that v-ATPase deficiency is the principal basis for autophagy deficits in PS-FAD and is a major contributor to abnormal calcium homeostasis - a second well-established γ-secretase independent deficit resulting from PS1 loss of function. Restoration of lysosomal function and autophagy in PS1-deficient cells by re-acidifying lysosomes suggests novel approaches to therapy for AD and possibly other neurodegenerative conditions. Supported by the National Institute on Aging.

Disclosures: J. Lee: None. **D. Wolfe:** None. **M. McBrayer:** None. **L. Haslett:** None. **A. Kumar:** None. **Y. Sato:** None. **P. Mohan:** None. **E. Coffey:** None. **C. Mitchell:** None. **E. Lloyd-Evans:** None. **R.A. Nixon:** None.

Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.08/I2

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: CALM impacts on Aβ42 production ratio through modulation of clathrin-mediated endocytosis of γ-secretase

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Abstract: Alzheimer disease (AD) is the most common type of dementia worldwide. Several lines of evidence suggest that aberrant production, clearance and aggregation of amyloid-β peptide (Aβ) in brain are linked to the etiology of AD. Recently, genome-wide association studies (GWASs) in late-onset AD patients have reported evidence that variation in phosphatidylinositol binding clathrin assembly protein (PICALM) gene confers a genetic risk for AD. A following meta-analysis using GWAS data has revealed that these variants in PICALM showed strong genetic interaction with another risk gene APOE ε4, suggesting that PICALM could be involved in Aβ metabolism in brains. PICALM gene encodes a protein which has a PtdIns(4,5)P2-binding AP180 N-Terminal Homology (ANTH) domain at its N terminus, along with several AP2/clathrin binding motifs in the C-terminal region, indicating that CALM functions in the initial step of clathrin-mediated endocytosis through the proper formation of

clathrin coated pits on the cell surface. To investigate the role of CALM in Aβ generation, we analyzed the effects of depletion of CALM on the processing of amyloid precursor protein (APP). Intriguingly, the ratio of Aβ42, the most aggregable Aβ species, was decreased in CALM-depleted culture cells as well as in brains of Picalm+/- mice compared to those in wild-type mice. In addition, we found that γ-secretase is constitutively endocytosed via clathrin-mediated pathway, and the endocytosis of γ-secretase was significantly blocked in CALM-depleted cells. We also showed that recombinant CALM ANTH domain binds nicastrin, which is an essential component for γ-secretase. In summary, these data suggest that alteration in the rate of clathrin-mediated endocytosis by the loss of CALM affects the steady-state localization of γ-secretase, which consequently impacts on the production of pathogenic Aβ42.

Disclosures: K. Kanatsu: None. **Y. Morohashi:** None. **T. Watanabe:** None. **T. Tomita:** None. **T. Iwatsubo:** None.

Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.09/I3

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Alzheimer-Forschung-Initiative Grant 11811

BMBF KNDD Grant 01GI1004B

Title: Generation of a faithful model for familial Alzheimer’s disease with presenilin-1 mutations

Authors: V. KURTH[1], I. OGOREK[1], T. JUMPERTZ[1], C. U. PIETRZIK[2], J. LOPEZ-RIOS[3], *S. WEGGEN[1];
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Abstract: Background: The vast majority of familial Alzheimer’s disease (FAD) cases with autosomal-dominant inheritance harbor heterozygous mutations in the presenilin-1 (PSEN1) gene. PSEN1 mutations increase the proportion of the aggregation-prone Abeta42 peptide species. In addition, PSEN1 mutations have been proposed to impair crucial cellular processes including signal transduction and calcium homeostasis. However, these effects and their potential contribution to the clinical phenotype of FAD remain controversial, likely due to the

use of overexpression models that did not accurately reflect the genetic background and the biochemical composition of the gamma-secretase complex in FAD patients.
Objectives: To incorporate specific mutations into the endogenous PSEN1 gene in mouse embryonic stem (ES) cells by an innovative gene-targeting strategy termed dual recombinase-mediated cassette exchange (dRMCE).
Methods: dRMCE takes advantage of pre-targeted mouse alleles such as those generated by the International Knockout Mouse Consortium (IKMC). In these conditional alleles, the target sites for Cre and Flp recombinases can be exploited to re-engineer the genomic locus with high frequency.
Results: To introduce mutations into the conditional PSEN1 allele, we have generated replacement constructs encompassing exons 5-12 corresponding to 75% of the mouse PSEN1 protein flanked by FRT and loxP recombination sites. These constructs were co-transfected with a vector encoding both Flpo and iCre into ES cells containing the PSEN1 conditional allele. For constructs encoding either wild type or mutant PSEN1, extensive validation by PCR demonstrated successful replacement of the conditional allele with a frequency of approximately 30%. To verify equal expression of both the re-engineered PSEN1 allele and the second unmodified allele, we compared the levels of PSEN1 protein in cell clones that had undergone successful recombination with negative cell clones that contained only one functional PSEN1 allele. Densitometry analysis demonstrated that the PSEN1 expression was twice as high in positive as compared to negative cell clones, indicating that the replaced locus was fully functional and expressed normal levels of a correctly spliced PSEN1 mRNA.
Conclusions: The lack of consensus concerning the effects of PSEN mutations indicates the need for improved cellular models that account for the heterozygous expression of PSEN mutants. Our approach based on ES cells and genome editing is a cost-effective alternative to primary or iPS cells, and provides an FAD model suitable for stringently controlled biochemical and kinetic experiments.

Disclosures: V. Kurth: None. **I. Ogorek:** None. **T. Jumpertz:** None. **C.U. Pietrzik:** None. **J. Lopez-Rios:** None. **S. Weggen:** None.

Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.10/I4

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: National Institute On Aging R01AG033016

National Institute On Aging 1R01AG025952

Title: GGA3 deletion accelerates bace1 elevation and anxiety-like phenotype in 5XFAD mice

Authors: *W. KIM, K. R. WALKER, E. L. KANG, J. DONG, P. HAYDON, G. TESCO; Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Amyloid plaques are one of the neuropathological hallmarks of Alzheimer's disease (AD) and are mainly composed of the amyloid beta peptide (Aβ), which is derived by sequential proteolytic cleavage of amyloid precursor protein (APP). Beta-Site amyloid precursor protein cleaving enzyme 1 (BACE1) initiates the generation of Aβ and is upregulated in AD brains. We have previously shown that the trafficking molecule, GGA3, plays a key role in the degradation of BACE1 regulating its trafficking to the lysosomes. More importantly we have found that levels of BACE1 in AD brains are increased and inversely correlated with GGA3 levels. Next, we have demonstrated the role of GGA3 in the regulation of BACE1 *in vivo* by showing that BACE1 levels are increased in the brain of GGA3 null mice on a congenic background. In this study, we crossed GGA3 knockout mice with a mouse model of amyloid deposition expressing five familial AD (FAD) mutations.(5XFAD mice) to determine the effects of GGA3 deletion on BACE1 levels, Aβ accumulation and behavioral deficits. Previous studies have shown that BACE1 levels are significantly increased in the cortex of 6 month-old 5xFAD mice compared to non-transgenic mice. Given that GGA3 deletion elevated BACE1 protein levels *in vivo* GGA3-/-5XFAD bigenic mice would be expected to show a significant increase in BACE1 levels before reaching the age of 6 months. Therefore, we analyzed BACE1 levels in the hippocampus and cortex of 4-month-old GGA3+/+5XFAD, GGA3+/-5XFAD and GGA3-/-5XFAD mice.
As expected, we found that BACE1 levels were increased in the hippocampus and cortex of 4 month-old GGA3-/-5XFAD mice when compared with GGA3+/+5XFAD mice or non-transgenic mice. Next, the behavioral phenotype of GGA3+/+, GGA3+/-, GGA3-/-, GGA3+/+5XFAD, GGA3+/-5XFAD and GGA3-/-5XFAD mice was assessed using a battery of tests including open field, elevated plus maze, Y-maze and contextual fear conditioning both at 4 and 7 months of age. While we did not find a significant difference among all groups in 4 month old mice, 7 month-old GGA3-/-5XFAD bigenic mice showed reduced anxiety-like behavior in the elevated plus maze compared to GGA3+/+5XFAD, GGA3-/- or GGA3+/+ mice. In conclusion, our data indicate that GGA3 deletion accelerates BACE1 elevation and anxiety-like phenotype in 5XFAD mice. We are currently investigating whether elevated BACE1 levels lead to increased Aβ deposition in GGA3-/-5XFAD mice both at 4 and 7 months of age.

Disclosures: W. Kim: None. **K.R. Walker:** None. **E.L. Kang:** None. **J. Dong:** None. **P. Haydon:** None. **G. Tesco:** None.

Poster

525. **Beta and Gamma Secretase, BACE, and Presenilin**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 525.11/I5

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG032784

NIH Grant ES016774

VA Merit Review RX000331

Title: Pyroglutamate abeta are actively produced by cathepsin B beta-secretase in a transgenic Alzheimer's disease mouse model and neuronal-like chromaffin cells

Authors: *G. R. HOOK[1], J. YU[2,3], M. KINDY[2,3,4], V. HOOK[5];
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Abstract: Pyroglutamate amyloid-beta peptides (pyroGluAbeta) lacking the first two N-terminal amino acids of full-length amyloid-beta 40 and 42 peptides and having a cyclized N-terminal glutamic acid (pyroGluAbeta3-40/42) may be the cause of Alzheimer's disease (AD) by catalyzing the formation of highly toxic amyloid-beta (Abeta) oligomers. Until now, the only means for reducing pyroGluAbeta production was to inhibit the enzyme glutaminyl cyclase, which catalyzes the cyclization of glutamic acid. In theory, beta-secretase could also be a potential upstream target but as yet neither the primary beta-secretase, BACE1, nor the alternative beta-secretase, cathepsin B (CatB), have been investigated for potential roles in producing pyroGluAbeta3-40/42. We show that deleting the CatB gene reduces pyroGluAbeta3-40/42, and overexpression of CatB increases pyroGluAbeta3-40/42 in APPLon mice. Importantly, that CatB deletion also improved memory deficits in those animals. But BACE1 gene deletion had no effect on pyroGluAbeta3-40/42 or memory in those mice. The APPLon AD mice were selected because they express the human amyloid precursor protein (APP) containing the wild-type (wt) beta- and London mutant gamma secretase site sequences, allowing CatB cleavage of the wt beta-site sequence that is expressed in most AD patients. The mice were also designed to express isoform APP-695 because brain neurons only express APP-695, Cat B beta-secretase activity occurs in neurons, and most amyloid-beta in AD patients is from brain neurons. In addition, we show that neuronal-like bovine cells, which have human wt APP processing, secrete pyroGluAbeta3-40 via constitutive and regulated secretion but secretory vesicles of the regulated secretory pathway produces most of the pyroGluAbeta3-40 and that the CatB inhibitor CA-074Me reduces pyroGluAbeta3-40 undergoing regulated secretion. These data strongly argue that CatB actively produces pyroGluAbeta3-40 and pyroGluAbeta3-42 and supports

pyroGluAbeta3-42 initiating the nucleation of Abeta oligomers in neurons that leads to neurotoxicity in AD. Interestingly, others recently concluded the opposite (Demattos 2012 Neuron 76:908). A likely reason for that is the transgene APP used in those other animals was not expressed by brain neurons and thus not available for CatB processing and CatB production of pyroGluAbeta3-40.42 could not occur. The important conclusions here are that CatB is a new target for reducing pyroGluAbeta3-40 and pyrogluAbeta3-42 and CatB inhibitors will thereby likely be effective AD therapeutics.

Disclosures: G.R. Hook: A. Employment/Salary (full or part-time):; American Life Science Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Science Pharmaceuticals. **J. Yu:** A. Employment/Salary (full or part-time):; Applied Neurotechnology, Inc. **M. Kindy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Applied Neurotechnology, Inc. **V. Hook:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); American Life Sciences.**Poster**

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.01/I6

Topic: C.04. Parkinson’s Disease

Support: Robert and Ruth Halperin Foundation

Peggy K Cahill Family Research Foundation

Title: Incidence and modulation of resting state subthalamic nucleus beta rhythm in Parkinson's disease

Authors: *L. A. SHREVE[1], A. VELISAR[1], N. M. SHANIDZE[1], B. C. HILL[1], C. KILBANE[1], J. M. HENDERSON[2], H. YU[2], H. BRONTE-STEWART[1];
[1]Neurol. and Neurolog. Sci., Stanford Univ. Sch. of Med., Stanford, CA; [2]Neurosurg., Stanford Hosp. and Clinics, Stanford, CA

Abstract: Beta frequency neural synchrony in the Local Field Potential (LFP), recorded from the subthalamic deep brain stimulation (STN DBS) lead may characterize the resting state in Parkinson’s disease (PD) and may be a useful signal for demand based DBS. However, some rest recordings failed to show increased LFP beta power, for unclear reasons. Objective: To determine the incidence of beta synchrony in PD rest recordings. Methods: Rest LFPs were

recorded from the STN DBS lead intra-operatively, after lead placement in 124 STNs from 62 PD patients. Concurrent inspection for any patient movement was performed via angular velocity recordings from contralateral limbs (arm and leg), accelerometer recordings from the head, continuous video recordings encompassing the full body and intra-operative notes. Raw data was scrutinized for artifacts, and each rest LFP spectrogram was aligned with the motion analysis and video recordings. Power spectral peaks were classified as being in the theta (5- <8 Hz), alpha (8- <13 Hz) and beta (13- <35 Hz) bands. Results: Mean pre-operative UPDRS III off (on) medication - 39.80 (20.67) +/- 11.98 (10.72) (+/- sd). 13 recordings were excluded due to artifact. Of the remaining 111, 105 (94.6%) revealed increased beta band power at rest. Of the 6 in which no beta peak was evident, 4 had clear evidence of concurrent consistent resting tremor and had peaks in the theta and/or alpha range, 1 of 6 had intermittent tremor and movement, and 1 of 6 had frequent movements that clinically appeared to be dyskinesias. Many rest recordings had evidence of intermittent tremor and/or movement, during which beta band power was transiently attenuated. These included 37 sides with intermittent tremor, 18 sides with voluntary or involuntary movement, and 13 sides with tremor and movement. Conclusions: In a large number of unmedicated PD patients with concurrent objective monitoring of movement, beta band synchrony was evident in 94.6% of STNs. In 4 of 6 recordings without beta synchrony, concurrent consistent tremor was evident along with LFP peaks in theta and/or alpha bands. In 2 of 6 there was intermittent tremor and/or dyskinesias. We suggest that tremor and/or movement may have modulated a resting beta band as we observed many cases where intermittent tremor or movement transiently attenuated underlying beta band synchrony. This data supports the hypothesis that beta band synchrony characterizes the true resting state of the STN in unmedicated PD. Care should be taken however to monitor the patient as involuntary or voluntary movements may modulate resting beta rhythm.

Disclosures: L.A. Shreve: None. **A. Velisar:** None. **N.M. Shanidze:** None. **B.C. Hill:** None. **C. Kilbane:** None. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical and Nevro Corp.. F. Consulting Fees (e.g., advisory boards); Intelect Medical and Nevro Corp.. **H. Yu:** None. **H. Bronte-Stewart:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.02/I7

Topic: C.04. Parkinson’s Disease

Support: Robert and Ruth Halperin Foundation

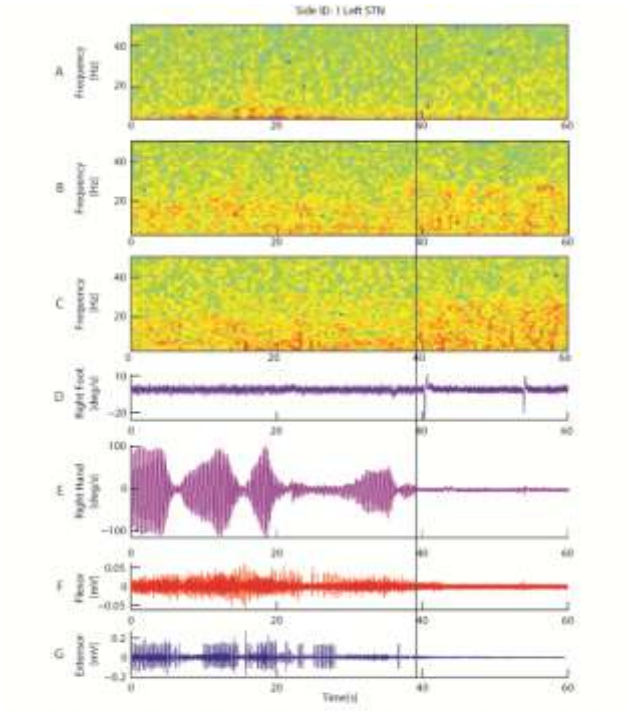
John A. Blume Foundation

Helen M. Cahill Award for Research in Parkinson's Disease

Title: Resolution of rest tremor reveals underlying subthalamic nucleus beta band synchrony in Parkinson's disease

Authors: A. VELISAR[1], L. A. SHREVE[1], B. C. HILL[1], H. YU[2], J. M. HENDERSON[2], *H. BRONTE-STEWART[1];
[1]Dept. of Neurol., [2]Dept. of Neurosurg., Stanford Univ., Stanford, CA

Abstract: While previous studies of local field potentials (LFPs) from the subthalamic nucleus (STN) during resting tremor in PD patients have revealed increased neural synchrony in low (5-13 Hz) frequency bands, we have occasionally noticed a reduction in the beta (13-35 Hz) band in this type of patient. Is the beta rhythm (13 - < 35Hz) is "missing" from the "rest" spectrum in tremor dominant PD subjects? Objective: to determine if tremor dominant PD is characterized by more beta frequency neural synchrony in periods of rest without tremor compared to tremor periods. Methods: LFP recordings from the deep brain stimulation (DBS) lead were made in 5 STNs from 3 PD subjects during which resting tremor resolved during the rest recordings. Concurrent movements/muscle activity of the contralateral limbs and the head were recorded using an accelerometer placed on the patient forehead, two angular velocity sensors placed on the hand and foot, and surface EMG electrodes placed on the wrist flexor and extensor muscles. The experiment was videotaped to check for movements not captured by the motion sensors and to confirm the presence or the cessation of tremor. Beta band power changes in the rest relative to tremor state were observed in spectrograms calculated using Short Fourier Transform with a moving window of 1 s long and 50 % overlap. The resulting frequency resolution was 1 Hz. Results: In all five recordings the resolution of tremor activity was associated with an emergence of power in the beta frequency band. Beta band power was greater in the rest no-tremor state than in the resting tremor state. Conclusions: resting tremor in PD modulates an underlying rest beta band rhythm. The dynamic modulation of the PD resting beta rhythm by rest tremor suggests that demand based DBS devices of the future would need to be programmed to respond to more than resting beta band power.



Beta rhythm emergence after hand tremor cessation, the vertical line shows the change in behavior state :
A) spectrogram of bipolar recording between contact s 2 and 3 of a DBS lead implanted in the left STN ;
B) spectrogram of bipolar recording between contact s 1 and 2 of a DBS lead implanted in the left STN ;
C) spectrogram of bipolar recording between contact s 0 and 1 of a DBS lead implanted in the left STN ;
D and E) angular velocity measurement of the right foot and hand;
F and E) muscle activity of the right wrist extensor and flexor

Disclosures: A. Velisar: None. **L.A. Shreve:** None. **B.C. Hill:** None. **H. Yu:** None. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical and Nevro Corp.. F. Consulting Fees (e.g., advisory boards); Intelect Medical and Nevro Corp.. **H. Bronte-Stewart:** None.

Poster

526. Parkinson's Disease: Human Studies Imaging

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.03/I8

Topic: C.04. Parkinson’s Disease

Title: Structural abnormalities of grey matter regions in Parkinson’s disease at baseline and 8-years follow-up

Authors: *C. WANG[1], L. CHAN[2], E. TAN[1,3], J. ZHOU[1];
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Abstract: Introduction
Parkinson's disease (PD) is a progressive neuronal degenerative disorder characterized by neuron loss and changes in brain microstructure. Previous studies showed loss in grey matter volume (GMV) and disrupted white matter integrity in brain regions extending beyond nigrostriatal pathway[1,2,3]. The goal of this longitudinal study is to investigate brain structural changes in PD with a focus to delineate specific features of neuronal degeneration in PD progression from that of normal aging process, measured by both GMV and white matter integrity.

Methods
Whole brain T1 images and diffusion (12 directions) images were acquired from 39 cognitive intact PD patients and 52 age-matched healthy controls (HC) at baseline in 2005 with 8-years follow-up.
Subject-level GMV probability maps were extracted from T1 images and analyzed using voxel-based morphometry (VBM)[4]. Fraction anisotropy (FA) and mean diffusivity (MD) were obtained from preprocessed diffusion images[5] and analyzed using VBM and automated anatomical labeling (AAL) based regions of interest (ROIs) method[6] to evaluate group difference as well as longitudinal changes. Age, gender and ethnicity were entered as nuisance variables.

Results
At baseline, among the 90 ROIs, we found increased MD in PD group in orbital part of left inferior frontal gyrus ($P<0.05$, FWE corrected). This finding was further supported using VBM approach on whole-brain MD maps ($P<0.001$, extent threshold corrected).
At 8-years follow-up, using ROI approach, we found increased MD in more extensive brain regions, including orbital part of bilateral inferior frontal gyrus, bilateral superior temporal pole ($P<0.05$, FWE corrected), as well as regions in insula and ventral medial frontal gyrus ($P<0.005$, uncorrected). Consistent reduced FA in PD was identified in most regions where MD was increased ($P<0.05$, uncorrected). ROIs and VBM approaches showed converging results. Moreover, reduced GMV in PD was found in similar regions of increased MD, including left inferior frontal gyrus ($P<0.05$, FWE corrected) and right inferior frontal gyrus, bilateral temporal lobe and bilateral insula ($P<0.001$, extent threshold corrected).

Conclusion
Compared with healthy elderly, we found converging evidence of increased MD, decreased FA and reduced GMV of PD group in a more extensive brain network, including prefrontal cortex, insula, and temporal regions after 8 years follow up. Our result suggests higher rate of neuronal degeneration in the specific network in PD compared normal aging process.

Ref
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Disclosures: C. Wang: None. **L. Chan:** None. **J. Zhou:** None. **E. Tan:** None.

Poster

526. Parkinson's Disease: Human Studies Imaging

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.04/I9

Topic: C.04. Parkinson's Disease

Support: NINDS R01 NS069779 (to PAS)

Title: Synchronization of globus pallidus neurons to cortical oscillatory activity in humans with Parkinson's disease and primary dystonia

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Abstract: Introduction: Parkinson's disease (PD) is characterized by excessive synchronization of neuronal activity throughout the basal ganglia thalamocortical loop, constraining neurons in an inflexible pattern of activity. Similar abnormalities in synchronization have also been proposed for primary dystonia, but its pathophysiology is less well characterized.Methods: We investigated synchronization between basal ganglia and cortex by simultaneously recording globus pallidus unit discharge with cortical local field potentials (LFPs) in patients undergoing pallidal deep brain stimulator insertion for PD or primary dystonia in the awake state, at rest. Cortical LFPs were recorded over primary motor cortex, primary sensory cortex, and the superior parietal lobule using a 6 contact subdural electrocorticography strip electrode. Synchronization was assessed by pallidal spike timed averages of cortical LFPs and of cortical broadband gamma band activity.
Results: We recorded 31 pallidal units in 6 pts with PD and 33 units in 7 patients with primary dystonia. Seventeen pallidal units in PD and 21 pallidal units in primary dystonia were significantly synchronized to at least one cortical recording site. In dystonia, the predominant cortical site for synchronization was parietal cortex, while in PD, the predominant site was primary motor cortex. Prominent synchronization was observed at frequencies throughout the lower frequency range examined (4-50 Hz) for both disorders. Additionally, broadband gamma

amplitude (50-200 Hz) also varied significantly as a function of pallidal spike times for many cells, but patterns across cells were variable. This spike-locked broadband gamma activity was itself significantly coupled to lower frequency rhythms (4-50 Hz) in many cases (19 pallidal units in PD and 12 pallidal units in dystonia).
Conclusions: We have shown that marked synchronization exists between pallidal neurons and cortical LFPs in two movement disorders. The topography of cortex-basal ganglia synchronization may explain specific manifestations of movement disorders. In dystonia, excessive synchronization of the basal ganglia to parietal lobe structures could account for well-described abnormalities in sensory function, including two point discrimination and mental rotation. Whereas, in PD, the excessive synchronization to motor cortex may prevent dynamic chances in oscillatory activity necessary for movement initiation, resulting in akinesia or bradykinesia.

Disclosures: N.C. Swann: None. **C. De Hemptinne:** None. **E. Ryapolova-Webb:** None. **J. Ostrem:** None. **P. Starr:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.05/I10

Topic: C.04. Parkinson’s Disease

Support: R01 NS075012

R01 NS052318

Title: STN-Cortex functional connectivity in de novo pd, moderate pd, et, msap, and psp

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Abstract: Resting state fMRI is used in the current study to measure low frequency fluctuations in the blood oxygen level dependent signal to identify differences in functional connectivity (FC) between STN and primary motor cortex. The study included 20 healthy controls, 20 de novo Parkinson disease (PD), 19 moderate PD, 14 essential tremor (ET), 13 multiple system atrophy

(MSAp) and 10 progressive supranuclear palsy (PSP) subjects. All subjects taking medication were tested after overnight withdrawal from medication. Voxel-wise analyses compared each group with controls to identify significant clusters ($P < .05$, corrected) of difference. We placed a seed in the left subthalamic nucleus (STN) and drew a specific region of interest (ROI) in the ipsilateral primary motor cortex to focus on this area. De novo PD and moderate PD subjects both had significant clusters of increased FC between the left STN and the ipsilateral primary motor cortex. The finding of a strong correlation with ipsilateral primary motor cortex for moderate PD is consistent with a study by Beaudrexel et al. (2011), and we have extended this to de novo PD. We also found that the motor section of the Unified Parkinson's Disease Rating Scale was positively correlated with STN-motor cortex FC in subjects with PD ($r = 0.41$, $p < .009$). Within the ipsilateral primary motor cortex ROI, each group of subjects had elevated FC compared with controls (P's < .05, uncorrected). Moreover, there were significant clusters of increased FC in the SMA, contralateral primary motor cortex, and ipsilateral primary motor cortex for MSAp subjects, and increased FC in the contralateral primary motor cortex of PSP subjects. Significant clusters of reduced FC were found in the ipsilateral middle frontal gyrus for de novo PD, increased FC in the contralateral middle occipital gyrus for ET, decreased FC in the contralateral precuneus for MSAp, and decreased FC in the contralateral middle frontal gyrus for PSP. All subject groups also showed significant clusters of reduced FC in the contralateral medial frontal gyrus/cingulate. These results provide new findings that include: 1) ipsilateral STN-motor cortex FC is increased in de novo PD, ET, MSAp, and PSP, 2) MSAp and PSP have increased contralateral STN-motor cortex FC, and 3) STN-lateral prefrontal cortex FC is decreased in all forms of parkinsonism whereas the ET subjects has abnormal STN-occipital cortex FC. These results demonstrate the importance of the STN in regulating motor function in PD and highlight potential differences in the STN-cortical connectivity among PD, ET and atypical parkinsonisms.

Disclosures: A.S. Kurani: None. **R.D. Seidler:** None. **C.M. Comella:** None. **D.M. Corcos:** None. **M.S. Okun:** None. **N.R. McFarland:** None. **D.E. Vaillancourt:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.06/I11

Topic: C.04. Parkinson's Disease

Support: NINDS Intramural Program

Title: Resting-state functional network reorganization in Parkinson's disease

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Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder associated with motor and cognitive symptoms caused in part by dopaminergic denervation in frontostriatal circuits. Difficulty performing self-initiated tasks is characteristic of PD patients.
Implementation of self-initiated plans requires three steps, each facilitated by different resting state (rs) neural networks: 1) detection of motivational cues by the salience network (SN), attention through the frontoparietal network (FPN) to guide behavior, and execution of the motor plan by the sensorimotor network (SMN). We hypothesized that these networks will exhibit deficits in PD.
36 PD patients off dopaminergic medication for at least 12 hrs (age: 62.0 ± 8.0, 12 females, Hoehn & Yahr: 2.42 ± 0.5 (mild bilateral disease with mild imbalance)) and 36 age- and gender-matched healthy volunteers (HV) (age: 61.1 ± 7.0) were scanned.
T1-weighted anatomical images and functional MRI during rest with eyes closed (TR: 2s, TE: 30ms, flip angle: 70, FoV: 240, voxel size: 3.75x3.75x5mm) were collected in a 3T scanner for 6 min and 50 s. Functional images underwent standard preprocessing. Nuisance variables were regressed out. The signal was bandpass filtered (0.01-0.08 Hz).
SN, FPN, and SMN nodes were constructed using coordinates from previous studies (Seeley 2007 and Dosenbach 2010). BOLD time courses were extracted from each node and correlated with all other nodes in the network. Graph metrics including node connectivity strength (NS), betweenness centrality (BC) and global/local efficiency (GE/LE) were calculated using the Brain Connectivity Toolbox. Between-group comparisons are reported at $p < 0.05$ (2-sample t-test).
HV > PD results: 1) higher NS in all networks (SN: paracingulate, left frontal pole, and right ventrolateral prefrontal cortex (vlPFC); SMN: bilateral postcentral gyrus and right posterior insula (PI); FPN: right dorsal and ventral PFC, and right superior parietal), 2) higher BC (SN: left supplementary motor area (SMA) and pre-SMA; SMN: right PI; FPN: left posterior parietal), 3) higher GE in SMN and FPN.
PD > HV results: 1) higher LE (FPN: left dorsolateral premotor cortex (dlPMC)), 2) higher BC (SMN: right vlPMC).
Overall, PD patients off medication exhibit weaker rs-functional connectivity between key nodes in salience, attention, and execution networks, and less efficient functional integration of information in attention and execution networks. Of note, mesial premotor areas (SMA/pre-SMA) involved in self-initiated tasks seem to play a bridging role across networks in HV. In PD, this role is shifted to lateral premotor areas that are normally activated in externally-guided tasks.

Disclosures: P. Lauro: None. **S. Tinaz:** None. **P. Malone:** None. **C. Lungu:** None. **S. Horovitz:** None. **M. Hallett:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.07/I12

Topic: C.04. Parkinson's Disease

Title: Spatial patterns of diffusion tensor imaging in Parkinson's disease

Authors: *S.-J. LIN[1,2], J. KOK[3], A. GRAG[3], M. F. BEG[3], M. J. MCKEOWN[1,2];
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Abstract: Background:
Diffusion Tensor Imaging is a way to assess white matter integrity. While Parkinson's disease (PD) is normally associated with pathology in the basal ganglia, previous reports have suggested changes in fractional anisotropy (FA) values in frontal regions. Here we propose using independent component analysis (ICA) to examine the independent spatial patterns associated with FA changes in PD.
Methods:
Eighteen subjects (5 females; 13 males; average age 63.56; daily L-dopa dosage 400-1200 mg) with PD were included in this study. All patients signed the informed consent prior to beginning of the study. Anatomical and diffusion scans were performed in a Philips Achieva 3.0 Tesla MRI scanner at UBC Hospital. The anatomical image was a 3D T1-weighted scan with CLEAR homogeneity correction, covering a 256×170×200mm^3 field of view with a 256×170×256 voxel image. The diffusion weighted scans consisted of 32 scans with varying gradients and one B0 scan. The scans covered a 212×132×212 field of view with a 256×60×256 image, using a 96×96 scan matrix. Images were registered to a one image using a high-dimensional registration process. The FA data were smoothed with a 6x6mm kernel and then decomposed by the fastICA package in Matlab. The loadings on the spatial patterns across subjects were then correlated with clinical indices.
Results:
After correction for multiple comparisons, two subject-specific ICA loadings of the spatial components significantly correlated with clinical indices. One component, largely located over the primary motor cortex, demonstrated a positive correlation with daily L-dopa dosage. Another ICA component was significantly correlated with overall UPDRS scores, and the associated spatial map was located in the caudal brainstem.
Conclusions:
Our results suggest that FA values are significantly affected in Parkinson's disease is spatially-

specific patterns. The changes in the motor cortex suggest that requirements for greater medication may be dependent upon microstructural changes in the motor cortex in PD.

Disclosures: S. Lin: None. **J. Kok:** None. **A. Grag:** None. **M.F. Beg:** None. **M.J. McKeown:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.08/I13

Topic: C.04. Parkinson’s Disease

Support: NIH RC4 073008

5 T32 AG 258-14

Title: Functional connectivity changes in early-stage Parkinson's disease

Authors: *P. BOORD, E. COLLINS, M. ASKREN, T. J. GRABOWSKI, Jr;
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Abstract: Introduction
Cognitive impairment (CI) in Parkinson's disease (PD) is often present at the time of initial diagnosis; becomes more frequent and pronounced in later stages of disease; and commonly culminates in dementia. The basis for this impairment, however, remains unclear. Functional connectivity (FC) differences measured by functional MRI (fMRI) have been observed in PD in default and cortico-striatal networks, but FC differences in attention and salience networks have not been reported. We report here FC differences in these networks in early-stage PD, in the absence of behavioral differences and gray matter (GM) volume atrophy.
Method
Medicated, early-stage, cognitively normal PD patients (n=14, age=62.4, SD=8.7, most with right-lateralized weakness) were compared to healthy controls (HC; n=10, age=56.6, SD=7.0). Resting state fMRI (rsFMRI) was acquired for 12 mins with eyes open. The Attention Network Test (ANT; Fan et al 2002) was administered in six blocks of six minutes duration. Two scanning sessions were acquired in most subjects (n=23) and the statistical maps were averaged across sessions prior to higher order analysis.

rsFMRI mean time courses were extracted from seed regions in the default mode (DMN), dorsal attention (DAN; Power et al 2011) and salience (SAL; Menon and Uddin 2010) networks. Seed-based FC maps were z-transformed. GM volume was estimated using a T1 MPRAGE image and voxel-based morphometry (VBM). PD and HC were compared for differences in FC and GM volume using higher order analysis, with statistical maps thresholded at the cluster level (pFWE-corr < 0.05; extent threshold > 50 voxels).

Results

A widespread FC reduction was observed in PD in the medial frontal wall relative to left and right anterior intraparietal sulcus (aIPS) and anterior cingulate. FC was also reduced between left aIPS and left caudate, and right frontal insular cortex and cuneus/precuneus. No significant reductions in FC were observed in the DMN. No differences in attention measures (alerting, orienting, executive) were observed between groups in the ANT. GM volume reductions were observed in PD, chiefly in left frontoparietal cortex, with significance at the set level (8 clusters; $p = 0.015$), but not at individual clusters.

Conclusion

Significant reductions in FC can be observed in early-stage PD, even in the absence of significant reductions in GM or changes in attention measures. FC reductions between frontal cortex, DAN and SAL might underlie CI observed in later stages of PD, and these connectivity patterns may serve as useful biomarkers to test and develop treatments for cognitive decline in PD. Future work in our lab will aim to investigate these proposals.

Disclosures: P. Boord: None. **E. Collins:** None. **M. Askren:** None. **T.J. Grabowski:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.09/I14

Topic: C.04. Parkinson’s Disease

Support: University of Michigan, School of Kinesiology

Gustavus and Louise Pfeiffer Foundation (Rachael D. Seidler)

Title: Cerebellar resting state functional connectivity in Parkinson’s patients on and off medication

Authors: *S. FESTINI[1], J. A. BERNARD[6], Y. KWAK[7], S. PELTIER[2], N. I. BOHNEN[3], M. L. T. M. MULLER[3], P. DAYALU[4], R. D. SEIDLER[5];
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Abstract: Parkinson's disease is a progressive neurodegenerative disorder associated with both motor and cognitive impairments. We have previously reported altered resting state functional connectivity between the basal ganglia and the cerebral cortex in Parkinson's disease, including hyperconnectivity in Parkinson's patients OFF medication compared to healthy controls that is down-regulated by L-DOPA medication (Kwak et al., 2010). Although the basal ganglia are a primary target of degeneration in Parkinson's disease, other structural and functional brain changes involving the cerebellum have been observed. For instance, it has been suggested that Parkinson's patients may recruit cerebellar networks to compensate for striatal degeneration and dopamine depletion (e.g., Palmer et al., 2010). Moreover, Liu et al. (2013) found evidence for altered resting state functional connectivity of the dentate nucleus of the cerebellum in Parkinson's disease, with decreased cortical default mode connectivity and increased compensatory cerebellar-cerebellar connectivity.

We extend this research by implementing a lobule-based cerebellar resting state functional connectivity analysis (see also Bernard et al., 2012) in Parkinson's patients and by comparing connectivity in patients ON and OFF their medication and healthy controls. A lobule-based approach allows us to examine connectivity between anatomically disparate regions of the cerebellum. Twenty-five Parkinson's patients and twenty-three controls of similar age and gender completed structural and functional magnetic resonance imaging (MRI) scans. Functional scans were collected at rest, while participants viewed a fixation cross for 8 minutes. Outside the scanner all participants completed a battery of motor and cognitive assessments.

Results indicate greater cerebellar functional connectivity in Parkinson's patients OFF medication than ON medication between Right Lobule VI and areas such as the precuneus and the supramarginal gyrus. Additionally, results indicate hypoconnectivity in Parkinson's patients ON medication compared to healthy controls. For example, there is evidence for reduced cerebellar functional connectivity with Right Crus I and the bilateral middle frontal gyrus, with Right Crus II and the bilateral middle frontal gyrus and the precentral gyrus, and with Right Lobule VI and the precentral gyrus, postcentral gyrus, inferior parietal, and superior temporal regions. We will further explore the relationships between cerebellar resting state functional connectivity and behavior to determine if the observed effects are pathological or compensatory.

Disclosures: S. Festini: None. **J.A. Bernard:** None. **Y. Kwak:** None. **S. Peltier:** None. **N.I. Bohnen:** None. **M.L.T.M. Muller:** None. **P. Dayalu:** None. **R.D. Seidler:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.10/I15

Topic: C.04. Parkinson's Disease

Support: NSFC Grant 30370473

NSFC Grant 30770476

NSFC Grant 81171061

Title: Neuronal oscillation patterns in the subthalamic nucleus and the ventrolateral thalamus in patients with Parkinson's disease

Authors: *P. ZHUANG[1], M. HALLETT[2], S. GUO[1], L. HE[1], Y. ZHANG[1], J. LI[1], Y. LI[1]; [1]Xuanwu Hosp, Capital Med. Uni, Beijing, China; [2]HMCS, NINDS, NIH, Bethesda, MD

Abstract:

Background: Increased oscillatory firing and synchrony between neurons within the basal ganglia and thalamus appear to play a key role in the pathophysiology of Parkinson's disease (PD).

Objective: To explore oscillatory activity in the STN and the Vop/Vim in patients with PD.

Methods: Forty-three PD patients (male: 28; female: 15; age 57.6 ±10.3 years) were studied. All patients had tremor, rigidity and bradykinesia. 23 patients underwent implantation of a deep brain stimulation (DBS) electrode into the STN and 20 patients underwent lesioning or implantation of a DBS electrode into the Vop/Vim. Microelectrode recordings were performed in the STN or Vop/Vim, and the EMG of the body side contralateral to surgery was recorded simultaneously. Single units were identified. The interspike interval (ISI) and coefficient of variation of ISI were calculated. Oscillatory patterns were evaluated using power spectral analysis with the Welch method.

Results: A total of 178 neurons were identified from the STN and 190 neurons were identified from the Vop/Vim. Spectral analysis of these neurons showed that there were three types of neuronal activity: (1) neurons with tremor frequency oscillations which displayed burst activity at tremor frequency concurrently with limb tremor. Their mean oscillation frequency was 4.2±0.7 Hz (with a range of 3.8-6 Hz), defined as the tremor frequency band oscillation (TFB) neurons. (2) Neurons with high-frequency oscillations (>8 Hz). Their mean oscillation frequency were 21.5±8.6 Hz (with range of 8-30 Hz), defined as the β frequency band oscillation (βFB) neurons. (3) Neurons with no oscillatory activity, defined as non-oscillation neurons. Few neurons (>30 Hz) displaying oscillatory activity in the gamma band were not further analyzed in the present study. Of all STN neurons with oscillations (n=59), 29% neurons were with TFB oscillation and 71% neurons were with βFB oscillation. Oppositely, of total Vop/Vim neurons with oscillations (n=31), 77% neurons had TFB oscillation whereas 23% neurons had βFB oscillation. Further analysis found that the density of neurons with TFB oscillation was almost twice in Vim than that in

Vop ($p<0.05$). Furthermore, a majority of neurons with βFB oscillation (20 of 25) was found in Vop.
Conclusion: Neurons with βFB oscillation were observed in the STN and Vop (presumed pallidal relay nucleus) supporting the prediction that the basal ganglia is involved in parkinsonian bradykinesia/rigidity. Neurons with TFB oscillations were mainly localized in the Vim (presumed cerebellar relay nucleus) supporting the view that the Vim is involved in tremor generation.

Disclosures: P. Zhuang: None. **M. Hallett:** None. **S. Guo:** None. **L. He:** None. **Y. Zhang:** None. **J. Li:** None. **Y. Li:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.11/I16

Topic: C.04. Parkinson's Disease

Title: Differences in dopaminergic disruption between dementia with Lewy bodies and Parkinson's disease

Authors: *K. OKITA[1], K. ISHII[2], K. ODA[2], M. SAKATA[2], J. TOYOHARA[2], M. IYO[4], K. KANEMARU[5], S. MURAYAMA[3], K. ISHIWATA[2];
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Abstract: Objective: Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are neurodegenerative disorders sharing common pathological feature of Lewy bodies. We characterized the difference in the nigrostriatal degeneration patterns between these diseases presenting equivalent parkinsonism severity. Further, we investigated the relationships between dopaminergic dysfunction and motor and cognitive dysfunction severity.
Methods: We studied 28 PD patients, 22 DLB patients, and 8 healthy subjects selected based on diagnostic guidelines with additional inclusion/exclusion criteria. Further, we incorporated neuroimaging findings. Motor and cognitive symptoms were scored using the unified Parkinson's disease rating scale and Mini-Mental State Examination(MMSE). PET scans were performed using ^{11}C-labeled 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane (^{11}C-CFT) and ^{11}C-labeled raclopride (^{11}C-RAC) as indices of pre- and post-synaptic dopaminergic functions,

respectively.
Results: In the caudate and anterior putamen, significantly lower binding of ^{11}C-CFT was observed in DLB than in PD in all Hoehn and Yahr (H&Y) stages. In the posterior putamen, the binding of ^{11}C-CFT was significantly lower in DLB in the H&Y stage 2. In contrast, there is no significant difference binding of ^{11}C-RAC between these diseases in both of caudate and putamen. A significantly lower caudate-to-putamen ratio of ^{11}C-CFT binding was observed in DLB than in PD ($p < 0.05$). A positive correlation between MMSE and ^{11}C-CFT binding was observed in the caudate among PD and DLB patients ($\rho = 0.54$, $p < 0.001$).
Conclusion: Thus, more severe dopaminergic dysfunction was shown in DLB patients than in PD patients with the same level of motor dysfunction, suggesting modulatory mechanisms of motor symptoms in DLB. Further, dopaminergic function in the caudate is likely associated with cognitive impairment of PD and DLB subjects.

Disclosures: K. Okita: None. **K. Ishii:** None. **K. Oda:** None. **M. Sakata:** None. **J. Toyohara:** None. **M. Iyo:** None. **K. Kanemaru:** None. **S. Murayama:** None. **K. Ishiwata:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.12/I17

Topic: C.04. Parkinson’s Disease

Support: CSRA Parkinson Support Group Grant PSG00026B

Title: Parkinson’s disease patients: Plasma cytokine profile

Authors: *C. WAKADE[1], J. MORGAN[2], R. LUCAS[3], S. SRIDHAR[3], R. SUHAG[4], R. CHONG[5];
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Abstract: Neuroinflammation plays a crucial role in the pathogenesis of Parkinson’s disease (PD). The systemic inflammation is known to contribute to the ongoing neurodegeneration in PD and is also a sequel to PD symptoms in early and late stages (1). Pro and anti-inflammatory cytokines levels may associate with the severity of the PD symptoms. We examined the plasma levels of thirteen cytokines in PD patients, age-matched controls, and young healthy controls: GM-CSF, IFN-γ, IL-10, IL-12, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, and TNF-α. Milliplex MAP kit was utilized to measure these cytokines using human cytokine magnetic beads at 0.08 - 1.01 pg/mL sensitivity. Moderate PD patients demonstrated significantly higher

levels of IL-4 compared to all elderly controls (p=0.016). IL-6 was higher in moderate PD patients compared to young healthy controls (p=0.026). Levels of IL-7 were higher in moderate PD patients (p=0.002), both mild and moderate PD patients (p=0.007) and all elderly controls (p=0.006) compared to young healthy controls. Similarly, all the PD patients demonstrated higher levels of IL-8 (p=0.047) and TNF-α (p=0.025) compared to all young healthy controls. In conclusion, cytokine levels in PD, particularly IL-7 need to be further explored using larger samples.

1) Perry VH. Innate inflammation in Parkinson's disease. Cold Spring Harb Perspect Med 2012;2:a009373.

Disclosures: C. Wakade: None. **J. Morgan:** None. **R. Lucas:** None. **S. Sridhar:** None. **R. Suhag:** None. **R. Chong:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.13/I18

Topic: C.04. Parkinson's Disease

Support: ANR

Title: Amazing astrocytic activation in parkinsonism

Authors: *G. CHARRON[1], E. DOUDNIKOFF[2], N. SOLARI[2], M.-H. CANRON[2], J. BAUFRETON[2], C. VEGA-ROÏATTI[2], E. BOUE-GRABOT[2], S. OLIET[3], E. BEZARD[2]; [1]IMN CNRS UMR 5293, Bordeaux, France; [2]IMN UMR 5293, Bordeaux, France; [3]Neurocentre Magendie INSERM U862, Bordeaux, France

Abstract: Dopamine (DA) deprivation in Parkinson's disease (PD) provokes complex and multiple changes in striatal DA target neurons that contribute to development of characteristic motor disorders such as bradykinesia, rigidity and resting tremor and later on of dyskinesia. The mechanisms triggering and/or contributing to the morphological and functional modifications affecting the basal ganglia (BG) network remain elusive and we consider too premature the current efforts without gaining an intimate knowledge of the whole synaptic organization at the striato-pallidal level. Puzzlingly enough, the role of astrocytes in such complex pathological changes has almost remained untouched. The current concept of BG organization and function excludes (so far) the most numerous cells in the brain, i.e. the astrocytes. For decades, astrocytes have been regarded as passive partners of neurons in the central nervous system, but this view has been challenged recently and they are now integrated in the concept of "tripartite synapse",

the structure consisting of pre- and post-synaptic elements of the synapse and an associated astrocyte process, supporting the idea that astrocytes participate actively in information processing. Gliotransmission includes the release of many chemical transmitters from astrocytes, including classical transmitters (glutamate, GABA, ATP and D-serine), peptides and cytokines using diverse mode of release (vesicular and non-vesicular). Although the past decade has seen an explosion of research on role of neuron-astrocyte interactions in the control of brain function, the role(s) of astrocyte in PD and LID remains to be investigated. Thus, the present work, undertaken in three animal models of PD as well as in human PD brains: (i) unravels the yet unknown plastic astrocytose in the parkinsonian and dyskinetic basal ganglia (ii) in the absence of gross variation in gliotransmitter levels; characterizes the connectivity of the astrocytes through expression of the connexins (iii) between themselves and (iv) with cortico-striatal and striatopallidal synapses.

Disclosures: G. Charron: None. **E. Doudnikoff:** None. **N. Solari:** None. **M. Canron:** None. **J. baufreton:** None. **C. Vega-Roïatti:** None. **E. Boue-Grabot:** None. **E. Bezard:** None. **S. Oliet:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.14/J1

Topic: C.04. Parkinson’s Disease

Support: NIH Grant 078327

Title: Neurotoxic insults upregulate Prokineticin-2 levels in Parkinson’s disease models and in PD patients

Authors: M. L. NEAL, R. GORDON, H. JIN, V. ANANTHARAM, *A. G. KANTHASAMY, A. KANTHASAMY;
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Abstract: Prokineticin 2 (PK2) is a secreted chemokine-like protein that mainly functions in the brain for olfactory bulb biogenesis and circadian rhythms. We previously demonstrated that PK2 is up regulated in dopaminergic neurons upon neurotoxic insults, as determined by targeted qPCR array analysis. Additionally, administration of recombinant PK2 protected against MPP+-induced dopaminergic neuronal cell death, suggesting a pro-survival role of PK2 in dopaminergic neurons. In order to further define the role of PK2 in Parkinson’s disease (PD) neuropathology, herein we performed immunohistochemical and Western blot analyses of

postmortem midbrain tissues from human PD cases and age-matched control subjects. Moreover, quantitation of serum PK2 levels using ELISA revealed lower serum concentrations of PK2 in PD patients as compared with their respective controls. In contrast, no significant change in serum PK2 levels was observed between PD and control samples. In the next set of experiments, we sought to investigate the expression profile of PK2 in animal models of PD. Time-course analyses of PK2 in the MPTP-induced mouse model of PD showed a time-dependent up regulation of PK2 in the nigral tissues, with maximal PK2 levels achieved at 12 hr post-MPTP injection. Immunohistochemical studies further confirmed that MPTP-induced PK2 upregulation in the nigral dopaminergic neurons. We also used the PK2-eGFP GENSAT mouse model to characterize the expression level of PK2 following MPTP challenge. Our results showed a dramatic increase in PK2 expression in the MPTP-treated SN as compared to controls. At the cellular level, we also found that the PK2 receptors, PKR1 and PKR2, are expressed in both neuronal and glial cells. Functional studies revealed that stimulation of PK2 receptors induces an increase in intracellular Ca2+ and promotes glial migration and proliferation. Collectively, our findings provide a potentially novel correlation between PK2 signaling and dopaminergic neuronal survival in an MPTP model of PD and midbrains from PD patients. The results suggest that PK2 signaling may be pivotal in dopaminergic neuronal survival, thereby implicating this secretory protein in PD pathogenesis (supported by NIH grant 078327).

Disclosures: M.L. Neal: None. **H. Jin:** None. **A.G. Kanthasamy:** None. **R. Gordon:** None. **V. Anantharam:** None. **A. Kanthasamy:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.15/J2

Topic: C.04. Parkinson’s Disease

Support: MJFF

Title: Alpha-synuclein in the colonic submucosa of PD compared to MSA patients

Authors: *H. B. DODIYA, K. M. SHANNON, A. KESHAVARZIAN, J. H. KORDOWER; Neurosci. Dept., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Parkinson disease (PD) diagnosis depends on the clinical recognition of motor signs including tremors, rigidity and bradykinesia. But these motor symptoms do not appear until the advanced stage of degenerative processes into the mid-brain with at least 40-60% cell loss in the substantia nigra. With a potentially long premotor course, there is an urgent need to develop

premotor biomarkers. Recently, our group has demonstrated α-synuclein pathology in colon samples prior to onset of PD (2-5 years before first motor PD symptom). Our group has also shown α-synucleinopathy in the colonic submucosa samples of early untreated PD cases (n=10) compared to the healthy controls (n=23) and inflammatory bowel disease (IBD) patients (n=23), suggesting specificity of alpha synuclein pathology within the colon samples of PD cases compared to IBD and controls. To establish whether a similar pathological expression of α-synuclein occurs in the colon of other synucleinopathies, the current study compared α-synuclein expression in colon samples of PD to that of multiple system atrophy (MSA) and healthy controls. Like PD, MSA is a synucleinopathy although α-synuclein is principally a major component of oligodendroglia in this disease and not neurons. The occurrence of glial cell inclusions (GCIs) and the filamentous aggregation of α-synuclein in the neurons of several brain regions are potentially a synergistical process that may cause neuronal degeneration in MSA. In the current study, we collected biopsy of the distal sigmoid colon samples (PD: n=28, MSA: n=6, Controls: n=8) using unprepped flexible sigmoidoscopy procedure. Immunohistochemistry studies for α-synuclein were performed on these biopsy samples. From all PD cases (n=28), 18 showed α-synuclein staining in colonic submucosa. The presence and intensity of α-SYN staining was reviewed and rated in a blinded manner using 5 point scale system (Shannon KM, 2012). Using the 0-4 point scale (0 = no staining and 4 = robust staining), in PD (n=28): 10 cases had no staining, 4 cases had 1+, 2 cases had 2+, 5 cases had 3+ and 7 cases had 4+ scores. Compared to PD cases, in MSA (n=6): 5 had no staining, 1 had 1+, and none had 2+, 3+, or 4+ scores. While in Control (n=8): 4 had no staining, 4 had 1+ and none had 2+, 3+, 4+ scores. These data indicate that α-synuclein staining is specific for PD and not seen in other synucleionopathies such as MSA or in IBD (Shannon et al, 2012) or age-matched controls.

Disclosures: H.B. Dodiya: None. **J.H. Kordower:** None. **K.M. Shannon:** None. **A. Keshavarzian:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.16/J3

Topic: C.04. Parkinson’s Disease

Title: The NINDS Repository Biomarkers Discovery Collection: a public resource of biomaterials for neurodegenerative disease research

Authors: *M. J. SELF[1], K. GWINN[2], M. SUTHERLAND[2], C. A. PÉREZ[1], W. MUHAMMAD[1], G. M. BALABURSKI[1], J. GILROY[1], M. FRASIER[3], L. VINCENT[3], R. A.

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Abstract: Neurological disorders present a massive challenge to healthcare systems globally. Identification of novel and reliable biomarkers that would allow for more efficient diagnosis, monitoring of disease onset and progression, and assessment of response to therapeutics, holds immense promise for improving clinical outcomes in individuals affected with disorders such as Parkinson's Disease and Huntington's Disease. The National Institute of Neurological Disorders and Stroke (NINDS) Repository at the Coriell Institute for Medical Research, also known as the NINDS Human Genetics Resource Center, has an overall mission of accelerating discovery of causes and risks for neurological disease by sharing biomaterials and de-identified clinical data. As a centralized facility for storage, processing, and distribution of biofluids (cerebrospinal fluid, plasma, serum, whole-blood, urine) and nucleic acid (DNA and RNA), the NINDS Repository serves as an integral component in the effort to identify and validate biomarkers of neurological disease. The establishment of large collections of biological samples obtained longitudinally from both affected and neurologically healthy individuals should prove invaluable for furthering investigation of biochemical markers via transcriptomic, proteomic, or metabolomic approaches. Currently the NINDS Repository collects samples under multiple NINDS sponsored biomarker initiatives including the Parkinson's Disease Biomarkers Program (PDBP) and the Neurobiological Predictors of Huntington's Disease study (PREDICT-HD), as well as a jointly sponsored study (BioFIND) on Parkinson's Disease in collaboration with the Michael J. Fox Foundation. The NINDS Repository aims to: (i) ensure that samples collected for biomarker discovery are of premier quality by collaboratively establishing unified standards of sample collection; (ii) provide rapid feedback to clinical sites regarding sample appearance and quality; (iii) maintain secure, high quality sample storage conditions with real-time monitoring and recording systems; (iv) perform standardized laboratory processing and quality assurance using validated operating procedures. The NINDS Repository thus provides a vital resource for research designed to discover and validate biomarkers of neurological disorders. Biomarker discovery samples are available upon request either directly from the NINDS Repository web catalog (http://ccr.coriell.org/NINDS), or via NIH-sponsored resources with links to the online catalog.

Disclosures: M.J. Self: None. **K. Gwinn:** None. **M. Sutherland:** None. **C.A. Pérez:** None. **W. Muhammad:** None. **G.M. Balaburski:** None. **J. Gilroy:** None. **M. Frasier:** None. **L. Vincent:** None. **R.A. Corriveau:** None. **C. Tarn:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.17/J4

Topic: C.04. Parkinson's Disease

Support: Canadian Institutes of Health Research

Title: Cortical thinning and subcortical white matter changes in Parkinson's Disease

Authors: KOSHIMORI[1,2], B. SEGURA[1], L. CHRISTOPHER[1,2], A. E. LANG[2], S. HOULE[1], A. P. STRAFELLA[1,2];
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Abstract: Background: The pathology of Parkinson's disease (PD) involves widespread brain areas beyond the nigrostriatal dopamine pathway and produces motor and non-motor symptoms. Whole brain analysis is needed to understand the complexity of the disease. Structural neuroimaging studies have demonstrated changes in gray and white matter in PD patients: however, few studies have investigated both gray and white matter changes in the same group of the patients. Purpose: The present study investigated gray and white matter changes of whole brain in PD patients using MRI. Specifically, we compared cortical thickness, subcortical gray matter volume, and microstructure of white matter of a group of PD patients with those of healthy controls. Methods: Cortical thickness maps and the values of subcortical gray matter volume were derived from T1-weighted images using FreeSurfer. Fractional anisotropy (FA) and mean diffusivity (MD) maps were derived from DWI images using FSL. We also correlated each imaging data with severity of the motor symptoms measured using Unified Parkinson's Disease Rating Scale (UPDRS) III, duration of disease, and cognition measured by Montrreal Cognitive Assessment (MoCA) in PD group. Results: In the cortical analysis, PD group showed significant cortical thinning in left superiofrontal gyrus ($p < 0.032$, corrected) and postcentral extending anteriorly precentral and caudal middle frontal gyri ($p < 0.001$, corrected) compared with HC group. In the white matter analysis, PD group showed significantly higher MD values in multiple white matter tracts including bilateral inferior longitudinal fasciculi, bilateral uncinate fasciculi, forceps minor, bilateral anterior thalamic radiation, bilateral superior longitudinal fasciculi, body of the corpus callosum, and left corticospinal tract ($p < 0.05$, corrected) compared with HC group. Furthermore, in PD group, FA values showed significant negative correlation with disease duration in the left anterior thalamic radiation, body and part of splenium of corpus callosum, bilateral corticospinal tracts, left superior longitudinal fasciculus, and left inferior longitudinal fasciculus ($p < 0.05$, corrected). Conclusions: Our data demonstrate that PD patients show cortical and subcortical structural brain changes and white matter changes are associated with the disease duration.

Disclosures: Koshimori: None. **B. Segura:** None. **L. Christopher:** None. **A.E. Lang:** None. **S. Houle:** None. **A.P. Strafella:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.18/J5

Topic: C.04. Parkinson's Disease

Support: DFG KFO-219

Title: Cerebellar networks with basal ganglia: Degeneration of cerebello-pallidal and nigro-striatal projections in PD

Authors: E. A. PELZER[1], A. HINTZEN[1], C. MELZER[1], A. SCHÖNEBERGER[2], D. Y. VON CRAMON[1], L. TIMMERMANN[2], *M. TITTGEMEYER[1];
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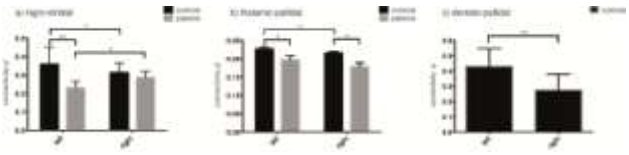
Abstract: Neuroanatomical studies using virus transneuronal tracers in macaque monkeys recently demonstrated that substantial interactions exist between basal ganglia and cerebellum (Bostan et al., 2013). To which extent these interactions are present in the human brain remains unclear; however, these connections are thought to provide an important framework for understanding cerebellar contributions to manifestation of basal ganglia disorders, especially with respect to pathogenesis in Parkinson's disease (PD; cf., Helmich, 2012; Wu and Hallet, 2013).
In this study we apply in vivo connectivity analysis following diffusion MRI and tractography to investigate pathological changes in PD in the dentato-pallidal and nigro-striatal pathways.
To this end, 3 T diffusion and structural MRI images were acquired in 12 right-handed, non-demented male PD patients (mixed type=6; akinetic-rigid =6) and 12 age-matched controls (mean age: 63 y). All patients underwent L-Dopa-therapy (LEDD: 678 mg/d, the UPDRS-III motor score revealed on average 16 points after a standardized levodopa dose of 200 mg, and on average 29 points without medication). All MR-images were analysed with FSL 5.0.2 (http://fsl.fmrib.ox.ac.uk/fsl/). Masks of dentate nucleus, pallidum, thalamus, caudate nucleus, putamen and substantia nigra were outlined in MNI standard space. Each mask was used as seed and target, respectively, for tractography; resulting connectivity values were statistically analysed in correlation to disease severity.
We find that: (1) diffusion MRI offers the possibility for an in vivo quantification of basal ganglia circuitry; (2) PD patients have reduced nigro-striatal connections; (3) significant

alterations in intrathalamic areas with projections from both cerebellar and pallidal pathways; (4) akinectic-rigid patients show a significant loss in dentato-pallidal connectivity, pointing towards a retrograde degeneration in these pathways.
This is one of the first studies indicating in vivo retrograde degeneration in basal ganglia satellite systems of PD patients.



Disclosures: E.A. Pelzer: None. **A. Hintzen:** None. **C. Melzer:** None. **A. Schöneberger:** None. **D.Y. von Cramon:** None. **M. Tittgemeyer:** None. **L. Timmermann:** None.

Poster

526. Parkinson's Disease: Human Studies Imaging

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.19/J6

Topic: C.04. Parkinson’s Disease

Support: NIH R01-NS-52318

NIH R01-NS-075012

Michael J. Fox Foundation for Parkinson’s Research

Title: Functional and structural neuroimaging of Parkinson’s disease and the parkinsonian variant of multiple system atrophy

Authors: *P. J. PLANETTA[1], P. SHUKLA[1], A. S. KURANI[3], D. M. CORCOS[4], C. L. COMELLA[5], N. R. MCFARLAND[2], M. S. OKUN[2], D. E. VAILLANCOURT[1];
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Abstract: Parkinson’s disease (PD) and the parkinsonian variant of multiple system atrophy (MSAp) are progressive neurodegenerative disorders that can be difficult to differentiate due to their overlapping motor features. While magnetic resonance imaging (MRI) techniques have revealed structural differences in PD and MSAp, the functional differences between these

diseases have not been assessed across the entire brain. To this end, the present study used structural MRI and task-based functional MRI (fMRI) at 3 T in 14 MSAp patients, 14 PD patients, and 14 age-matched controls. fMRI examined the blood oxygenation level-dependent (BOLD) signal during a precision grip force task that has been shown to activate the basal-ganglia-thalamo-cortical circuit in healthy individuals. Behaviorally, the MSAp group was slower to reach steady-state force amplitude than controls and both patient groups were slower to release force from steady-state amplitude back to baseline. Voxel-wise analyses were performed on the whole-brain and cerebellum normalized fMRI data using family wise error correction ($p <$ .05). The results showed that the PD group was hypoactive in the putamen, globus pallidus, thalamus, primary motor cortex, dorsal premotor area, and pre-supplementary motor area compared to controls. In the cerebellum, the PD group had reduced activity in lobule VI and crus I and increased activity in lobule V and crus I. Compared to controls, the MSAp group was also hypoactive in the putamen, globus pallidus, and thalamus, but showed only one other area of hypoactivity, in cerebellar lobule VI. In addition, the MSAp group was hyperactive in the inferior parietal lobule, lingual gyrus, and several cerebellar areas such as lobules VIIIa, V, VI, crus I, and vermis. When the PD and MSAp groups were compared directly, there were no BOLD activity differences in the basal ganglia or thalamus, but the MSAp group was hyperactive in primary sensorimotor cortex, dorsal and ventral premotor areas, insula, and throughout the cerebellum. In summary, this study showed that PD and MSAp have similar functional changes in the basal ganglia and thalamus and differential changes in the cortex and cerebellum during a precision grip force task. The PD group had reduced BOLD activity in the basal ganglia, thalamus, and cortex, and both reduced and increased activity in the cerebellum. The MSAp group also had reduced activity in the basal ganglia and thalamus, but increased activity in the cortex and cerebellum. Currently, we are performing voxel-based morphometry on the structural MR images to investigate how these functional changes relate to brain atrophy.

Disclosures: P.J. Planetta: None. **P. Shukla:** None. **A.S. Kurani:** None. **D.M. Corcos:** None. **C.L. Comella:** None. **N.R. McFarland:** None. **M.S. Okun:** None. **D.E. Vaillancourt:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.20/J7

Topic: C.04. Parkinson’s Disease

Title: The effects of MR field strength on connectivity-based segmentation of the SN/VTA using diffusion tensor imaging at 3 and 7-tesla

Authors: *M. BETTS[1], J. KAUFMANN[2], M. KANOWSKI[2], K. NEUMANN[1], P. SCHULZE[1], E. DÜZEL[1,3];
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Abstract: The dopaminergic SN/VTA plays a key role in numerous cognitive functions such as reward processing, learning and memory. There is much interest in the neuroimaging community to delineate subregions of the SN/VTA as this complex is differentially affected by both health (e.g. normal aging) and disease (Parkinson's disease). One approach is to use diffusion tensor imaging (DTI) with probalistic tractography to segment structures based on their known regional connectivity (Behrens & Berg 2005; Chowdhury et al., in press). In this study we aim to use DTI-derived connectivity maps to subdivide the SN/VTA into dorsomedial and ventrolateral subregions based on connectivity to the ventral and dorsal striatum respectively.
Using ultra high-field DTI at 7-tesla (T) in parallel with 3T DTI, we seek to determine the benefit of higher magnetic field strength with respect to nigro-striatal connectivity. Whilst signal-to-noise (SNR) for DWI-derived measures has previously been reported to be generally higher at 7T (Polders et al., 2011), higher field strengths may also be more prone to eddy current distortions, magnetic susceptibility gradients and signal loss, offsetting potential SNR advantages (Zhan et al., 2012).
Healthy young subjects were scanned at 3T using 30 (+ 1 b0 image) and 64 diffusion directions (+ 7 b0 images) at 1.8mm isotropic resolution using alternative diffusion gradient values and at 7T using 64 diffusion directions (+ 1 b0 image) at 1.4mm isotropic resolution, obtaining the average of two acquisitions. We examined how scanner field strength affects SNR of non-diffusion (b0) reference images, number of fibers, connectivity measures, multi-channel reconstruction methods (sum-of-squares and adaptive combined at 3T) and DTI-derived diffusion metrics. SN segmentation was performed using magnetization transfer, proton density and susceptibility weighted imaging to explore how each contrast may impact nigrostriatal connectivity.
At 3T, improved SNR in both the SN and striatal target regions was observed with increasing number of diffusion bvals and directions using adaptive-combined reconstruction. Whilst a decrease in SNR was observed at 7T, a substantial increase in fiber projections from the SN to the ventral and dorsal striatum was found suggesting an increase in nigrostriatal connectivity with respect to 3T. No differences in quantitative diffusion metrics were observed at higher field strength.
Our provisional findings suggest greater connectivity observed at higher resolution using 7T may be beneficial in the parcellation of the SN/VTA, to obtain a more fine-grained understanding of the pathological changes occurring in aging and disease.

Disclosures: M. Betts: None. **J. Kaufmann:** None. **K. Neumann:** None. **P. Schulze:** None. **E. Düzel:** None. **M. Kanowski:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.21/J8

Topic: C.04. Parkinson's Disease

Title: Characterizing PD tremor using smartphone measurements of patient daily behavior

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Abstract: Tremor is a common symptom of many movement disorders, including Parkinson's Disease (PD) and Huntington's Disease. Furthermore, the stage of a movement disorder is often tightly correlated with the severity of tremor. A key element, therefore, in understanding the severity of a movement disorder (and to help aid its treatment) is to quantify the dynamics of tremor symptoms. Such quantification can be used as feedback of the motor disorder severity, which can then supplement an appropriate treatment (such as patient specific treatments, quantifying the efficacy of treatments, telemedicine, etc.). In this study we quantify the symptoms of PD by developing computational tools based on dynamic measurements. We use a dataset provided by the Michael J. Fox Foundation that monitors daily behavior of PD patients and control subjects using a smartphone. Using machine learning techniques to classify activities with measured acceleration features, we are able to isolate and parse the various behaviors of the PD patients and control subjects (such as walking, remaining sedentary, etc). By isolating behaviors, we report our preliminary results on quantifying the relationship between tremor severity and the stage of PD using the Unified Parkinson's Disease Rating Scale (UPDRS). This study strongly supports the use of smartphones as an aid for clinical applications by capturing the daily behavior of patients.

Disclosures: M. Yazdani: None. **G.G. Gamble:** None. **W.C. Lennon:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.22/J9

Topic: C.04. Parkinson's Disease

Support: NIH R01 NS047388

NIH R01 NS059736

NIH NRSA T32 EB004314

NIH MSTP T32 GM007250

NIH CTSA TL1 TR000441

Title: Deep brain stimulation tractography-activation models developed with 7T MRI data

Authors: *K. GUNALAN[1], A. CHATURVEDI[1], Y. DUCHIN[2], G. SAPIRO[3], N. HAREL[2], C. MCINTYRE[1];
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Abstract: Subthalamic deep brain stimulation (DBS) is an established therapy for advanced Parkinson's disease. However, the underlying mechanisms of action and brain regions directly modulated by the stimulation are not well understood. Multiple lines of evidence show that axons within the vicinity of the active electrode contact are directly activated by stimulation. Magnetic resonance imaging-based techniques, known as tractography, enable definition of the trajectories of white matter pathways within the brain on a patient-specific basis. Therefore, we developed a novel methodology that combines DBS modeling with tractography to explicitly quantify the spatial extent of axonal activation due to the DBS electric field and visualize how these activated axons pathways project to different brain regions. These tractography-activation models (TAMs) enable comparison of the different activated pathways as the stimulation parameter settings are changed in a patient. In this study, we were specifically interested in DBS of 4 pathways in the subthalamic region: 1) subthalamopallidal, 2) pallidothalamic, 3) hyperdirect, and 4) cerebellothalamic. We investigated the relative activation of these pathways during unilateral DBS in a Parkinson's disease patient that had undergone preoperative, high-field (7T) structural and diffusion-weighted magnetic resonance imaging. FSL (FMRIB, England) was used to define the pathways, the voltage distribution for the stimulation settings was calculated using finite element modeling, and the axonal response to the corresponding extracellular voltages was simulated in multi-compartment axonal models with NEURON (Yale University, USA). Our results suggest that that individual pathways can be preferentially activated by TAM-based definition of the stimulation settings in the patient. Therefore, patient-specific TAMs developed with high-field MRI data have potential to enhance our understanding of the specific pathways responsible for therapeutic benefit from DBS.

Disclosures: K. Gunalan: None. **A. Chaturvedi:** None. **Y. Duchin:** None. **G. Sapiro:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Surgical Information Sciences, Inc. **N. Harel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Surgical Information Sciences, Inc. **C. McIntyre:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Surgical Information Sciences, Inc.. F. Consulting Fees (e.g., advisory boards); Boston Scientific Neuromodulation, Corp..

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.23/J10

Topic: C.04. Parkinson’s Disease

Support: NIH 1RC4NS073008-01

Title: Alterations of intrinsic functional connectivity in Parkinson’s disease

Authors: *T. MADHYASTHA[1], M. ASKREN[2], E. COLLINS[2], T. GRABOWSKI[2];
[1]Psychiatry and Behavioral Sci., [2]Univ. of Washington, Seattle, WA

Abstract: Correlations among low frequency spontaneous fluctuations in the blood oxygen level-dependent (BOLD) signal reflect the connectivity of intrinsic large-scale networks in the brain. Parkinson’s disease (PD) includes both motor and cognitive symptoms spanning memory and attentional domains and is characterized by systematic dopaminergic deficits. In the present study, we examine the differences between intrinsic functional connectivity in medicated early-stage PD patients (N=12, Mage=61) and healthy elderly controls (N=38, Mage=74) drawn from the Alzheimer’s Disease Neuroimaging Initiative.
We extract time-varying signals from regions of interest in attentional networks, the default mode network, and the salience network. We use a novel technique for characterizing network activity based on exploratory factor analysis of these variables in a structural equation modeling framework (ESEM) (Asparouhov & Muthén, 2009), implemented in Mplus. Within the ESEM framework the researcher can access typical SEM parameters, including standard errors, goodness of fit statistics and comparisons of competing models, and multiple group analysis. This allows rigorous comparison of the factor structure of the PD sample to that of healthy controls.
We find that the factor structure of the PD sample is significantly different than that of the

normal controls. Specifically, we observe coactivation of the anterior cingulate cortex, the posterior cingulate cortex, and right lateralized attention network nodes that is not present in controls. Differences in expression of the salience network during rest may reflect underlying dopaminergic dysfunction characteristic of PD or may be a consequence of treatments targeting the dopamine system. Dysregulation of the salience network over the course of disease may contribute to cognitive impairments observed in later stages of PD.

References

1. Asparouhov, T. & Muthén, B. Exploratory Structural Equation Modeling. Structural Equation Modeling: A Multidisciplinary Journal 16, 397-438 (2009).

Disclosures: T. Madhyastha: None. **M. Askren:** None. **T. Grabowski:** None. **E. Collins:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.24/J11

Topic: C.04. Parkinson’s Disease

Support: Barnes Jewish Hospital Foundation award

NIH grant NS075321

American Parkinson Disease Association

Title: Disruption of default mode network functional connectivity in Parkinson's disease

Authors: *M. C. CAMPBELL[1], J. M. KOLLER[2], E. R. FOSTER[3], A. Z. SNYDER[4], J. S. PERLMUTTER[1];
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Abstract: Introduction: Research on resting-state functional connectivity in Parkinson disease (PD) has primarily focused on cortico-striatal motor networks; however, cortical networks are also likely disrupted in PD given the pathological markers of cortical alpha-synuclein aggregation and cognitive decline associated with PD. Based on previous studies demonstrating that the default mode network (DMN) is sensitive to neurodegenerative diseases, we hypothesize DMN functional connectivity will be disrupted in PD and relate to cognitive function.
Methods: Resting-state BOLD scans (Seimens 3T Trio) were obtained from PD participants (N =

36) while off medication and from controls (CTRL; N = 28), matched for age and head movement. Participants with excessive movement were excluded from all analyses. Seed based functional connectivity analyses focused on the DMN regions. In addition, participants completed the Clinical Dementia Rating (CDR) evaluation and neuropsychological testing to determine cognitive function. All CTRLs had normal cognition (CDR =0); there were 17 PD with normal cognition (CDR = 0) and 19 PD with cognitive impairment (CDR = 0.5). Between-group analyses of resting-state functional connectivity first compared all PD and CTRL participants and then subgroup comparisons were conducted based on CDR rating. Fisher z-transformed correlation coefficients were extracted for significant seed-cluster pairs and correlated with cognitive performance and self-reported everyday cognitive function.
Results: PD participants demonstrated significantly lower DMN functional connectivity than the CTRLs between the lateral parietal lobe and the medial prefrontal cortex (mPFC) (ps <.05). There were no significant differences in DMN functional connectivity between PD with normal cognition and those with cognitive impairment. However, there were significant linear relationships between DMN functional connectivity and overall cognitive performance, executive function, visual-spatial function, and self-reports of everyday cognitive function (ps <.05). There were no significant relationships between DMN functional connectivity and disease severity.
Conclusion: These data demonstrate that the lateral parietal lobe loses functional connectivity with the mPFC for individuals with PD regardless of cognitive status. Furthermore, loss of DMN functional connectivity was related to cognitive performance and everyday cognitive function. These results suggest that the DMN functional connectivity is sensitive to even the earliest cognitive changes associated with PD.

Disclosures: M.C. Campbell: None. **J.M. Koller:** None. **E.R. Foster:** None. **A.Z. Snyder:** None. **J.S. Perlmutter:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.25/J12

Topic: C.04. Parkinson’s Disease

Support: Robert and Ruth Halperin Foundation

Title: Spectral distributions of STN local field potential in the beta band are more dynamic in movement compared to rest in subjects with Parkinson’s disease

Authors: *M. MILLER KOOP, H. BRONTE-STEWART;
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Abstract: Local Field Potential (LFP) recordings from the subthalamic nucleus (STN) from PD subjects at rest show beta band (13-35Hz) neural oscillations and synchrony. Studies have shown that beta band synchrony is stationary at rest and is attenuated around the onset of single movements. Until recently it was not known if this attenuation continued during a longer repetitive movement. Objective: to determine whether the resting state beta rhythm was modulated with continuous repetitive movement, and if the beta rhythm during continuous movement was stationary. Methods: LFPs were recorded intra-operatively from the deep brain stimulation lead (DBS, Medtronic 3389) immediately after its placement in the STN in 13 PD subjects, off medication for over 15 hours. Task: LFPs were recorded during 30s of rest and followed by 30s of continuous repetitive wrist flexion-extension (rWFE). Hand angular velocity was collected using angular velocity sensors. Only subjects that had a beta peak at rest between 13-35Hz, and who had at least 20% attenuation in total power in the 13-35Hz band during DBS (185Hz/2.5V or 130Hz/2V) were included. Results: Mean beta band power was not different between rest and movement epochs ($p>0.05$). However visual inspection showed that in all 13 subjects, power in the beta band decreased during early periods of the task up to about 4s. Rest and movement epochs within each subject were divided up into segments: early (0-10s) and late (20-30s). The power spectral density (PSD) (frequency resolution of 1Hz) of early movement (0-10s) was compared to that of late movement (20-30s) and similarly for the early and late rest segments. The number of 1Hz frequency bins that showed a significant difference between the early and late segments were determined for rest and movement (i.e. the 95 percent confidence intervals did not overlap). The total number of 1Hz frequency bins that showed a significant change in power was more than double between the movement epochs (38) compared to the rest (18) ($p<0.01$). This indicates that although in our cohort we did not determine a significant change in the mean power in the beta band between rest and movement epochs, we did see significant changes in the spectral distributions. This finding suggests that the movement beta band is more dynamic compared to the resting beta band. Whether this dynamic process is related to the quality of the movement is yet to be determined.

Disclosures: M. Miller Koop: None. **H. Bronte-Stewart:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.26/J13

Topic: C.04. Parkinson's Disease

Support: Robert and Ruth Halperin Foundation

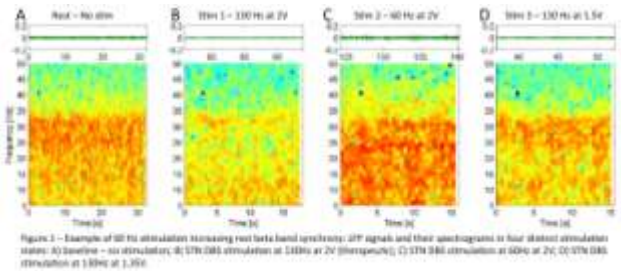
John A. Blume Foundation

Helen M. Cahill Award for Research in Parkinson's Disease

Title: Sixty hertz deep brain stimulation does not attenuate subthalamic nucleus beta rhythm in parkinson's disease

Authors: H. BRONTE-STEWART[1], L. A. SHREVE[1], B. C. HILL[1], H. YU[2], J. M. HENDERSON[2], *A. VELISAR[1];
[1]Neurol., [2]Neurosurg., Stanford Univ., Stanford, CA

Abstract: Objective: Subthalamic Nucleus deep brain stimulation (STN DBS) at frequencies of 130 to 185Hz at 2V reduces tremor, rigidity and bradykinesia in Parkinson's disease (PD) and attenuates resting STN local field potential (LFP) power in the 13 - 30 Hz range (beta band). STN DBS at frequencies below 100 Hz are not therapeutic except in a few cases for freezing of gait. We hypothesized that STN DBS at a low (non-therapeutic) frequency (60Hz, 2V) would not attenuate STN DBS resting beta band power (RBP).
Methods: During the experiments patients were awake and resting on the surgical bed. LFPs were recorded from electrodes 0-2 (Medtronic 3398 lead), at rest - no stimulation (30sec), and during (20 sec) randomly presented epochs of STN DBS through electrode 1, at 130Hz/2V (therapeutic), 130Hz/1.35V and 60Hz/2V, intra-operatively in 18 PD sides off medication, immediately after DBS lead placement and clinical testing. Beta band power was calculated between 13-30Hz using previously published methods.
Results: Recordings from five sides were discarded: two due to presence of artifacts, three due to different stimulation parameters. There was significant attenuation of RBP during therapeutic DBS ($P<0.01$) and during DBS at 130 Hz/1.35V ($P <0.025$) but not during 60Hz/2V DBS. The reduction in RBP was significantly greater at 130 Hz/2V compared to that at 60 Hz/2V ($P<0.01$). Although DBS at 60Hz/2V and 130 Hz/1.35V delivered the same total energy, only 130Hz/1.35V attenuated RBP. In 4 sides STN DBS at 60Hz/2V increased RBP. Conclusions: This is the first report of the effect of clinically determined non-therapeutic frequencies of DBS on RBP in the STN and demonstrates that low frequency (60Hz) does not change and in some cases increases RBP in PD. This may support the hypothesis that a mechanism of therapeutic DBS for PD is to attenuate beta band synchrony in sensorimotor networks.



Disclosures: H. Bronte-Stewart: None. **L.A. Shreve:** None. **B.C. Hill:** None. **H. Yu:** None. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical. F. Consulting Fees (e.g., advisory boards); Intelect Medical. **A. Velisar:** None.

Poster

526. Parkinson's Disease: Human Studies Imaging

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.27/J14

Topic: C.04. Parkinson's Disease

Support: Pilot Grant (JBW) from the Center for Gene Environment Studies in Parkinson's Disease (CGEP) at UCLA (NIH U54 ES016732),

Faculty Research Grant (JBW) from the UCLA Academic Senate's Committee on Research

NIH RO1 NS38992 (GWM)

Title: Full-length proteoforms of α-synuclein from human brain tissue by top-down mass spectrometry

Authors: *J. B. WATSON[1], T. A. SARAFIAN[1], C. M. RYAN[1], P. SOUDA[1], E. MASLIAH[4], U. K. KAR[1], H. V. VINTERS[2], G. W. MATHERN[3], K. F. FAULL[1], J. P. WHITELEGGE[1]; [1]Dept Psychiatry & Biobehav Sci., [2]Dept Pathology and Lab. Med., [3]Dept Neurosurg., David Geffen Sch. Med. UCLA, Los Angeles, CA; [4]Dept. of Neurosci. and Pathology, UCSD, La Jolla, CA

Abstract: The primary amino acid structure of full-length human α-synuclein in human brain surgically removed from a young patient (5.8 years old, female, ILAE Cortical Dysplasia I) was previously determined by combined liquid chromatography and top-down mass spectrometry [Sarafian TA, Ryan CM, Souda P, Masliah E, Kar UK, et al. (2013) Impairment of Mitochondria

in Adult Mouse Brain Overexpressing Predominantly Full-Length, N-Terminally Acetylated Human α-Synuclein. PLoS ONE 8(5): e63557. doi:10.1371/journal.pone.0063557]. N-terminal acetylation of α-synuclein was the only posttranslational modification (PTM) detected in protein from this tissue. The same isoform was also the only PTM detected in protein from brain mitochondria and cytosolic fractions from an overexpressing (ASOTg) early mouse model for Parkinson's Disease (PD), save for minor C-terminal truncations. An overexpressed full-length, N-terminally acetylated mostly monomeric form of human α-synuclein was sufficient to disrupt adult brain mitochondrial function in the ASOTg mice. Thus α-synuclein PTMs appear to occur infrequently in young human brain but may increase progressively with age in PD brain. New top-down mass spectrometric approaches are now in progress to examine the major proteoforms of α-synuclein in greater detail, and in additional resected brain samples, as well as in postmortem midbrain tissue from older PD, Diffuse Lewy Body Disease (DLBD), and DLBD plus Alzheimer's disease (AD) cases.

Disclosures: J.B. Watson: None. **T.A. Sarafian:** None. **C.M. Ryan:** None. **P. Souda:** None. **E. Masliah:** None. **U.K. Kar:** None. **H.V. Vinters:** None. **G.W. Mathern:** None. **K.F. Faull:** None. **J.P. Whitelegge:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.28/J15

Topic: C.04. Parkinson's Disease

Support: NIH R01 NS 32368 (CRF, P.I.)

Title: Human embryonic dopamine neurons transplanted into putamen of Parkinson patients survive for at least 22 years without immunosuppression

Authors: *C. R. FREED[1], R. E. BREEZE[2], W. M. ZAWADA[1], S. FAHN[3], D. EIDELBERG[4], S. JONES[1], W. ZHOU[1];
[1]Div. of Clin. Pharmacol., [2]Neurosurg., Univ. Colorado Sch. of Med., AURORA, CO; [3]Neurol., Columbia Presbyterian Med. Ctr., NEW YORK, NY; [4]Functional Brain Imaging, North Shore Univ. Hosp., Manhasset, NY

Abstract:
We have shown that embryonic dopamine cell transplants significantly improve objective signs of Parkinson's disease in direct relation to the preoperative response to L-dopa. We have now studied postmortem brain of 12 subjects who have died 7 months to 22 years after transplant.

Dopamine neurons were identified immunohistochemically in transplant tracks using a primary antibody to tyrosine hydroxylase and a secondary antibody labeled with fluorescent FITC. After identification of dopamine neurons by green fluorescence, pigment density was determined by measuring white light transmission through the cytoplasm of each cell. Twenty-five cells in each transplant track were measured. Pigment density increased linearly in transplanted dopamine neurons during the years after transplant. Parallel studies were performed on substantia nigra of children and adults who did not have Parkinson's disease. A similar age-related accumulation of pigment was seen. Despite the absence of immunosuppression at the time of surgery and in subsequent years, every transplanted fragment of human embryonic mesencephalon showed surviving dopamine neurons, indicating that the immune system did not destroy any transplant. While a few isolated Lewy-body like inclusions were occasionally observed, all transplants showed extensive fiber outgrowth into putamen, demonstrating that the transplanted dopamine neurons were physiologically robust. The total number of surviving dopamine neurons in each transplant site was not related to the years since transplant, showing that there was no progressive loss of neurons with time. The largest number of surviving dopamine neurons was found in a patient who died at age 85, ten years after transplant; therefore, patient age does not adversely affect survival of dopamine neurons.. We conclude that transplanted human dopamine neurons undergo morphologic evolution essentially identical to that seen in the normal substantia nigra. Our results indicate that the neurotrophic environment of the putamen of patients with idiopathic Parkinson's disease is normal since it can support long term development of transplanted dopamine neurons.

Disclosures: C.R. Freed: None. **R.E. Breeze:** None. **W.M. Zawada:** None. **D. Eidelberg:** None. **S. Fahn:** None. **S. Jones:** None. **W. Zhou:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.29/J16

Topic: C.04. Parkinson's Disease

Support: NIH Grant NS075321

Title: Correlation between altered cerebrospinal fluid levels of α-Synuclein and Aβ1-42 in Parkinson disease

Authors: *C. BUDDHALA, M. CAMPBELL, J. S. PERLMUTTER, P. T. KOTZBAUER; Neurol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Accumulation of misfolded α-synuclein (α-syn) protein in Lewy bodies and Lewy neurites is the defining pathologic feature of Parkinson disease (PD). The development of dementia in PD is consistently associated with neocortical α-syn accumulation. In our previous autopsy study, 60% of PD patients with dementia had neocortical accumulation of Aβ plaques. We investigated the metabolism of α-syn and Aβ1-42 in cerebrospinal fluid (CSF) to determine their utility as biomarkers for diagnosis and disease progression in PD. We measured baseline CSF levels of α-syn, Aβ1-42, total tau and phosphorylated tau in a cross-sectional cohort comprising idiopathic PD participants (n=60) and age-matched controls (n=20). CSF levels of α-syn and Aβ1-42 were significantly lower in PD (α-syn; p=0.0008, Aβ; p=0.0067) when compared to controls, but differences in CSF total tau and phosphorylated tau were minimal. In addition, when PD participants were stratified on the basis of clinical dementia rating (CDR) scale scores, the levels of CSF Aβ1-42 were lower in PD with mild cognitive impairment (CDR=0.5; n=23) (p=0.047) when compared to those without cognitive impairment (CDR=0; n=30). Moreover, CSF Aβ1-42 levels correlated inversely with mean cortical binding potential, as assessed by [C-11] - labeled Pittsburgh compound B amyloid imaging scans. There was also a strong positive correlation between CSF levels of α-syn and Aβ1-42 in PD participants ($p<0.001$) but not in controls, further supporting a connection between altered α-syn and Aβ metabolism in PD.

Disclosures: C. Buddhala: None. **M. Campbell:** None. **J.S. Perlmutter:** None. **P.T. Kotzbauer:** None.

Poster

526. **Parkinson's Disease: Human Studies Imaging**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 526.30/J17

Topic: C.04. Parkinson’s Disease

Support: Brain Research Trust Grant

Rosetrees Trust Grant

The Astor Foundation Grant

Title: The effect of subthalamic nucleus deep brain stimulation on effective connectivity within the basal ganglia motor loop

Authors: *J. KAHAN[1], M. URNER[2], A. MARREIROS[1], R. MORAN[3], L. ZRINZO[1], M. HARIZ[1], P. LIMOUSIN[1], K. FRISTON[4], T. FOLTYNIE[1];

[1]Sobell Dept. For Motor Neurosci. and Movement Disorders, UCL Inst. of Neurol., London, United Kingdom; [2]UCL Inst. of Cognitive Neurosci., London, United Kingdom; [3]Virginia Tech. Carilion Res. Inst., Roanoke, VA; [4]Wellcome Trust Ctr. for Neuroimaging, UCL, London, United Kingdom

Abstract: Starved of dopamine, the dynamics of the Parkinsonian brain result in a combination of symptoms impacting on both 'action' and 'resting' motor behaviour. Deep brain stimulation (DBS) has become an established means of managing these symptoms, although its mechanisms of action remain unclear. Dynamic causal modelling (DCM) of neuroimaging data can provide estimates of effective connectivity within and between regions of a network, furnishing predictions about how the observed neuroimaging data might have been generated. Original implementations of DCM regard the brain as a nonlinear deterministic system, requiring the experimenter to 'perturb' the system with exogenous inputs (experimental stimuli) in order to infer task-related effective connectivity. A recent extension ('stochastic DCM') incorporates random fluctuations into the equations governing the neuronal evolution of each region, freeing the system from deterministic assumptions, allowing the experimenter to model directed connectivity in the resting brain. In this work, we model the effective connectivity within the resting motor loop of the human brain in 12 patients with Parkinson's disease, with and without active bilateral subthalamic nucleus (STN) DBS, using resting state functional magnetic resonance imaging. Utilising a well-established, simplified model of the functional architecture of the motor cortico-striato-thalamic loop, we probe which connections, or combinations of connections are modulated by STN DBS to produce the observed BOLD signal recorded at rest. Using Bayesian model selection, we are able to demonstrate that a model incorporating DBS-related modulatory effects on the extrinsic direct, indirect, hyperdirect, thalamo-cortical, as well as cortico-striatal pathways, consistently outperforms equally plausible hypothesised models (posterior probability > 99%). Post-hoc model family analysis reiterates this further (posterior probability > 99%). Our findings highlight the disseminated effects of deep brain stimulation of the Parkinsonian resting motor network, specifically on the coupling between the cortex and basal ganglia, as well as between the nuclei of the basal ganglia. This work is an example of how researchers can use this method to provide a mechanistic insight into neurological/psychiatric diseases and their therapies in a non-invasive manner, without requiring the subject to complete a mentally demanding task. This work also provides a framework for analysing effective connectivity in resting state fMRI data with strong a priori hypotheses.

Disclosures: J. Kahan: None. **M. Urner:** None. **R. Moran:** None. **L. Zrinzo:** None. **M. Hariz:** None. **P. Limousin:** None. **K. Friston:** None. **T. Foltynie:** None. **A. Marreiros:** None.**Poster**

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.01/J18

Topic: C.04. Parkinson's Disease

Support: SFI Grant 10/RFP/ECE2720

Title: Application of temporally non-regular deep brain stimulation to a control theory model of the parkinsonian basal ganglia

Authors: *C. M. DAVIDSON[1], A. M. DE PAOR[1], M. M. LOWERY[2];
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Abstract: Deep Brain Stimulation (DBS) applied to either the subthalamic nucleus (STN) or the globus pallidus pars interna (GPi) effectively alleviates the motor symptoms of advanced Parkinson's disease that are no longer controllable with drug therapy alone. In Parkinson's disease dopamine loss leads to an increase in both the functional connectivity and oscillatory activity within the basal ganglia. Correlation between this oscillatory activity in the beta band (12-35Hz) and the motor symptoms of the disease has been established. Suppression of these oscillations by either drug therapy, DBS or a combination of both also correlates with an improvement in the motor symptoms of the disease. In current clinical practice, DBS consists of a regular, biphasic pulse train applied at frequencies in the 130-185Hz range, with the amplitude, pulse duration and frequency of stimulation adjusted for optimal patient improvement. In this study a computational mean-field model, which aims to capture the key physiological mechanisms of a group of interacting neurons in a mathematically tractable way, is used to represent the Parkinsonian basal ganglia. The effect of applying temporally non-regular stimulation to beta oscillations simulated using the computational model is examined. A number of different stimulation patterns are applied and the efficacy of each compared. Although they are specific to the model used, the results suggest that non-regular stimulation may provide greater suppression of oscillations than that achieved with regular stimulation, and therefore equivalent improvement in the motor symptoms would be predicted.

Disclosures: C.M. Davidson: None. **A.M. de Paor:** None. **M.M. Lowery:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.02/K1

Topic: C.04. Parkinson's Disease

Support: NIH Grant UL1TR000117

Title: The evolution of lead fixation techniques in deep brain surgery

Authors: *B. BROWN, C. VAN HORNE;
Dept. of Neurosurg., Univ. of Kentucky, Lexington, KY

Abstract: Deep brain stimulation (DBS) has demonstrated effective symptom relief for Parkinson's disease, essential tremor, dystonia, and may be an emerging treatment approach for Alzheimer's disease, depression, and obsessive-compulsive disorders. However, as with all device related procedures, complications are unavoidable. There are numerous reports in the literature regarding complications from DBS; however, there are few regarding complication prevention. In this study, the authors performed a review of published techniques for lead fixation as well as to present their anchoring technique. The novel lead fixation technique utilizes a silastic tubing to protect the lead, an injectable calcium phosphate cement (HydroSet®), and a titanium craniofacial miniplate. The HydroSet® sets rapidly, is non-toxic, is non-exothermic (as compared to methyl methacrylate), and stabilizes the lead prior to final fixation to the skull with the miniplate. The described technique has been utilized in 44 patients for implanted electrodes in the subthalmic nucleus, thalamus, or globus pallidus for a total of 66 lead placements without incidence of lead damage, migration, or infection.

Disclosures: B. Brown: A. Employment/Salary (full or part-time):; University of Kentucky. **C. van Horne:** Other; Medtronic.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.03/K2

Topic: C.04. Parkinson's Disease

Support: NIH R01 NS40894

Title: Optimized temporal patterns of deep brain stimulation reduce parkinsonian symptoms at low frequencies

Authors: *D. T. BROCKER[1], B. D. SWAN[1], R. Q. SO[1], D. A. TURNER[2], R. E. GROSS[4], W. M. GRILL[3];
[1]Dept. of Biomed. Engin., [2]Departments of Neurobio. and Surgery, [3]Departments of Biomed.

Engin. and Neurobio. and Surgery, Duke Univ., Durham, NC; [4]Departments of Neurosurg. and Neurol., Emory Univ., Atlanta, GA

Abstract: Deep brain stimulation (DBS) was developed empirically to use short (60-180 μs), high frequency (>100 Hz) pulses of electrical stimulation to ameliorate the motor symptoms of Parkinson's disease (PD). Current DBS systems deliver a regular pattern of stimulation (i.e., interpulse intervals do not vary across time), but the efficacy of DBS depends on the temporal pattern of stimulation, with random patterns of DBS exhibiting decreased efficacy, and some specific patterns exhibiting increased efficacy. We sought to exploit the dependence on temporal pattern and used model-based optimization to design a non-regular pattern of DBS for PD and subsequently tested this pattern in a hemi-parkinsonian rat model of PD and in human subjects with PD. We coupled a computational model of the PD basal ganglia with an engineering optimization technique (genetic algorithm) and designed a non-regular pattern of stimulation with a low average frequency of stimulation (45 Hz) and efficacy in the computational model similar to high frequency regular stimulation.

In hemi-parkinsonian rats and human subjects with PD, the optimized non-regular pattern of DBS was compared to high frequency regular stimulation, frequency-matched regular stimulation, and the stimulation-off condition. Performance was evaluated in the hemi-parkinsonian rats using two established methods for evaluating parkinsonian symptoms: methamphetamine-induced circling test and the bar test of akinesia. The optimized non-regular pattern of stimulation reduced parkinsonian motor symptoms in the rats more effectively than the stimulation off condition and frequency-matched regular stimulation. Bradykinesia and parkinsonian tremor were evaluated in human subjects with PD using an alternating finger tapping task and an accelerometer taped to the dorsum of the subjects hand, respectively. Again, the optimized non-regular pattern of stimulation reduced parkinsonian motor symptoms in the human subject more effectively than the stimulation off condition and frequency-matched regular stimulation. Finger tapping task performance was similar between the optimized 45 Hz pattern of stimulation and 185 Hz high frequency stimulation.

These results reinforce that temporal pattern is an important DBS parameter and provide evidence that non-regular patterns may be used to increase the efficiency of DBS for PD. The low average frequency of the optimized pattern of stimulation may reduce both stimulation-induced side effects and power consumption, thereby lengthening battery life and time between implantable pulse generator replacement surgeries.

Disclosures: D.T. Brocker: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Deep Brain Innovations, LLC. **B.D. Swan:** None. **R.Q. So:** None. **D.A. Turner:** F. Consulting Fees (e.g., advisory boards); Deep Brain Innovations, LLC. **R.E. Gross:** F. Consulting Fees (e.g., advisory boards); Deep Brain Innovations, LLC. **W.M. Grill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Deep Brain Innovations, LLC.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.04/K3

Topic: C.04. Parkinson's Disease

Title: Mobility in Parkinson's disease is improved through classical ballet-based instruction

Authors: *M. V. ALBERT, W. GOMEZ, A. MISKOVICK, C. LOPEZ-ORTIZ;
Rehab. Inst. of Chicago, Northwestern Univ., Chicago, IL

Abstract: Evidence is presented which indicates that classical ballet instruction, an enjoyable group activity, can improve motor symptoms in people with Parkinson's disease (PD). PD affects over four million adults throughout the world. Individuals with PD suffer from a number of impairments in movement and posture including tremor, rigidity, bradykinesia (slow movement), poor balance, and impaired gait. Exercise therapies have demonstrated significant improvements in PD symptoms, with dance classes increasingly being considered as a more enjoyable variant of exercise therapy. We present a pilot study of a classical ballet-based intervention involving five participants with PD. Uniquely to the design of this classical ballet PD class, each exercise was selected or adapted to counter a specific mobility deficit in Parkinson's disease; exercises emphasized mobility of each joint proximally to distally, coordination of lower and upper limbs as needed for a normal gait cycle, functional reaching, rhythmicity and phrasing in movement, and inclusion of principles from LSVT BIG therapy, all within the context of classical ballet technique. Subjects participated in a one-hour class twice per week over a period of eight months with a minimum attendance rate of 75%. Our mobility measures were the 10 meter walk test, the five times sit to stand test, the 360 degree turn test, and the composite Timed Up and Go (TUG) test calculated from the previous measures. All tests were performed at the beginning of the first and last class. Significant differences were found in the 10m walk test ($p<0.005$, paired t-test), the five times sit to stand test ($p<0.016$) and the composite TUG test ($p<0.004$). The timed 360 degree turn test demonstrated a decrease that was not statistically significant ($p >0.229$). To our knowledge, this pilot study is the first to report significant improvements in motor scores in a classical ballet-based dance class for subjects with PD.

Disclosures: M.V. Albert: None. **W. Gomez:** None. **A. Miskovick:** None. **C. Lopez-Ortiz:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.05/K4

Topic: C.04. Parkinson's Disease

Title: Effects of istradefylline alone and in combination with levodopa on motor and cognitive function in the MPTP-treated macaque model of Parkinson disease

Authors: *E. Y. PIOLI[1], Q. LI[1], J. YANG[1], A. CROSSMAN[1], E. BEZARD[2], J. S. SCHNEIDER[3];
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Abstract: Istradefylline has been suggested as an adjunct to L-dopa to decrease OFF time and to improve the ON period functioning in advanced Parkinson's disease (PD) patients experiencing L-dopa-related treatment complications. Little is known however about the effects of this drug, alone or in combination with L-dopa, on motor or cognitive symptoms in mild/moderate PD. Thus, the current was performed to evaluate effects of the adenosine A2A antagonist istradefylline, alone and as an adjunct to levodopa (L-dopa), on motor symptoms and cognitive functioning in a nonhuman primate Parkinson model. Six adult male cynomolgus macaque monkeys, previously trained to perform cognitive tasks (working memory, attention) were chronically administered MPTP until cognitive deficits appeared. After cognitive deficits were documented, MPTP administration continued with increasing doses until mild/moderate Parkinsonian motor symptoms emerged. Istradefylline was administered at 60, 75, and 100 mg/kg and motor and cognitive function were tested 50 min. later. L-dopa was administered at 2.5 - 25 mg/kg and was tested for effects on motor and cognitive functioning alone and in combination with istradefylline. Low doses of L-dopa partially improved motor symptoms and higher doses maximally improved motor symptoms. Istradefylline alone had no effect on motor symptoms and provided no additional benefit when added to either low or high doses of L-dopa. At low doses, istradefylline had no positive effect on working memory or attention and at higher doses tended to further disrupt at least some cognitive functions. In some animals, high dose L-dopa decreased commission errors on the attention test but at the expense of increasing omission errors. Istradefylline alone had no effect on attention. Addition of istradefylline to either low or high doses of L-dopa provided no benefit. Istradefylline shows little ability to improve motor or cognitive function, either alone or in combination with L-dopa, in a mild/moderate PD model that expresses both cognitive and motor deficits

Disclosures: **E.Y. Pioli:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **Q. Li:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **J. Yang:** A. Employment/Salary (full or part-time):; Motac Cognition. **A. Crossman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac Holding. **E. Bezard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac Holding. **J.S. Schneider:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac Cognition.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.06/K5

Topic: C.04. Parkinson's Disease

Title: Positive signals from biomarkers predict YKP10461 will be effective in Parkinson's disease

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Abstract: Type B monoamine oxidase (MAO_B) preferentially metabolizes phenylethylamine (PEA) and dopamine. The activity of MAO in human thrombocytes is almost exclusively type B and reflects brain MAO_B activity. MAO_B has been identified in human brain tissue with its activity increasing with age, and it has been studied widely in neuropsychiatric disorders. The inhibition of this enzyme is one of the important therapeutic strategies for the treatment of Parkinson's disease and other neurodegenerative diseases. MAO_A preferentially deaminates norepinephrine (NE) and serotonin. Dihydroxyphenylglycol (DHPG) is the metabolite of NE formed by this isozyme. Foods high in tyramine ingested along with a MAO_A inhibitor are known to increase blood pressure, sometimes to a dangerous degree, by displacing NE from intraneuronal stores. YKP10461 is a new chemical entity selected for the study of its potential in the treatment of PD because of its potent, selective, and reversible inhibition of MAO_B. (Park *et al*., 755.29. *Soc for Neurosci* 2012).
In the course of a Phase 1 first in human, single ascending dose study for safety and tolerance, pharmacokinetic (PK) parameters were obtained (Ciric *et al*., AAPS abstracts, 2013, in press). In this clinical study, 60 healthy subjects were enrolled at a single center: 10 subjects in each of 6 sequential cohorts. In each cohort, 7 subjects received oral doses of YKP10461 and 3 subjects

received placebo. The doses used were 10, 25, 50, 100, 200, and 250 mg. Blood samples from this study were analyzed for the presence of YKP10461 and, additionally, for MAO_B activity and levels of PEA and DHPG to assess the potential safety and efficacy in later proof of concept trials in PD.
The PK findings indicated that YKP10461 was orally available with C_{MAX} and AUC dose-proportional over all doses. Inhibition of MAO_B occurred in all cohorts and in all subject receiving YKP10461 compared
to subjects receiving placebo. Subjects dosed with YKP10461 showed elevation of PEA in all cohorts compared to placebo subjects. Only the first cohort (10 mg) and last cohort (250 mg) were analyzed for DHPG. No difference in DHPG levels was found between the dosed and placebo groups at either dose.
The findings regarding MAO_B and PEA demonstrate proof of pharmacology and suggest that efficacy studies may target doses lower than 10 mg. The finding of no change in DHPG levels strongly suggests that no inhibition of MAO_A occurred, signifying a lack of risk for tyramine-induced hypertensive crisis. In summary, YKP10461 has been confirmed in humans to be a potent and selective MAO_B inhibitor with potential efficacy and safety in the treatment of PD.

Disclosures: D.P. Taylor: A. Employment/Salary (full or part-time):; SK Life Science. **E. Graham:** A. Employment/Salary (full or part-time):; SK Life Science. **A. Pegan:** A. Employment/Salary (full or part-time):; SK Life Science. **H.W. Kim:** A. Employment/Salary (full or part-time):; SK Life Science. **W. Han:** A. Employment/Salary (full or part-time):; SK Life Science.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.07/K6

Topic: C.04. Parkinson’s Disease

Support: University of Kentucky start-up funds

NIH UL1TR000117

Title: Peripheral nerve graft implants into the substantia nigra of subjects with Parkinson’s disease undergoing deep brain stimulation surgery: A safety study

Authors: *J. E. QUINTERO[1], W. S. Z. ASFAHANI[2], F. ZAHEER[3], J. A. GURWELL[3], G. A. GERHARDT[4], J. T. SLEVIN[3], C. G. VAN HORNE[2];

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Abstract: In Parkinson's disease (PD), the substantia nigra undergoes a loss of dopaminergic cells and cell function that, in part, manifests into the outward symptoms of PD. One avenue of intense efforts to treat this disease involves the delivery of neurotrophic factors to restore cell function. Previous studies have shown that neurotrophic factors including GDNF, BDNF, and NT-3 can promote dopaminergic function. Continuous, targeted delivery of these growth factors to patients, however, has been fraught with complications and failures. An alternative source of neurotrophic factors may be Schwann cells from the peripheral nervous system. After injury, Schwann cells release a host of growth factors including GDNF, NGF, BDNF, and NT-3. We have begun a pilot study to examine the safety and feasibility of implanting an autologous peripheral nerve graft into the substantia nigra of PD patients undergoing deep brain stimulation (DBS) surgery. Multi-stage, DBS surgery targeting the subthalamic nucleus is performed using standard procedures. After the DBS leads are implanted, a section of sural nerve (approximately 5mm in length) containing Schwann cells is excised and unilaterally delivered, using a custom-designed cannula, into the area of the substantia nigra. While immediate post-operative magnetic resonance scans do not indicate evidence of tissue disruption, MR-images will be further examined at three and 12 months after surgery. Meanwhile, adverse events are being continuously monitored. Neuropsychological assessments conducted 12 months after the implant surgery will be compared to pre-surgery assessments. Subjects will undergo a unified Parkinson's disease rating scale (UPDRS) evaluation before surgery and at 1, 3, 6, 9, and 12 months after surgery. On-going evaluations will help assess the safety and feasibility of implanting peripheral nerve tissue in conjunction with DBS implant surgery for patients with PD.

Disclosures: J.E. Quintero: None. **W.S.Z. Asfahani:** None. **F. Zaheer:** None. **J.A. Gurwell:** None. **G.A. Gerhardt:** None. **J.T. Slevin:** None. **C.G. van Horne:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.08/K7

Topic: C.04. Parkinson's Disease

Title: Impedance reliability across neuromodulation devices during neurostimulator replacement

Authors: *E. L. HARGREAVES[1], R. J. DITOTA[1], S. WONG[2], S. F. DANISH[1]; [1]Neurosurg., [2]Neurol., Robert Wood Johnson Med. Sch., New Brunswick, NJ

Abstract: Deep Brain Stimulation (DBS) is an established adjunct neurosurgical treatment for movement disorders. At the heart of the DBS system is the neuromodulation device, which has a number of diagnostic capabilities to ensure the integrity of the DBS system. One of these capabilities is impedance testing. We have shown previously that Medtronic's most recent Activa (PC/SC) neurostimulators are more accurate in identifying the impedance of known resistors during bench tests than their Soletra predecessors. Further we have shown that the accuracy of all devices declines as the battery charge wears down, particularly for resistances outside the clinical range. Regardless, Soletras and the models from the Activa family within the clinical range of 1000 Ohms are typically accurate to within 5%, which can increase to 10% as the battery declines. All neurostimulator models at this impedance tend to overestimate the impedance. Here, we compare the impedance assessments of Soletras about to be deactivated to the impedance assessments of their newly implanted replacement Activa SCs. The Soletras were replaced in pairs, and as such, a number of the devices were considered functioning at a normal battery charge, with only their contralateral counterparts failing. Thus, the discrepancy between the device impedance assessments was examined. Nine individuals predominantly implanted in the subthalamic nucleus (STN), but also one in the ventral intermediate nucleus of the thalamus (VIM), and one in the globus pallidus interna (GPi), underwent replacement of 18 devices, of which 16 had sufficient charge to perform impedance assessments. Consistent with our previous bench tests, the results indicate that the Activa SCs systematically estimated the impedance to be 7% lower than that estimated by the Soletras (a mean difference of 63 Ohms; sem 20.73). If the differences between the Soletras and Activa SCs were grouped according to the battery charge of the Soletras to be replaced (failing <3.67V vs. functioning >3.69V) then these differences diminished from 8.7% to 4.9% respectively. Further, battery charge correlated positively with the difference in the failing Soletra grouping (r=.49), while battery charge was uncorrelated in the functioning Soletra grouping (r=.08). We interpret this to suggest that the spread of Battery charges and impedance differences were diminished in the functioning Soletra group, just as the spread was exaccerbated in the failing Soletra group. Finally, these reported differences exceed those recorded from simple same device (Activa PC) transient deactivations.

Disclosures: E.L. Hargreaves: None. **R.J. DiTota:** A. Employment/Salary (full or part-time):; Medtronic. **S. Wong:** None. **S.F. Danish:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.09/K8

Topic: C.04. Parkinson's Disease

Title: Monitoring the effects of subthalamic nucleus deep brain stimulation on sensorimotor cortex and peripheral muscles

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Abstract: Background
Deep brain stimulation (DBS) has been used for controlling motor symptoms of Parkinson's disease. Optimizing the DBS parameters using objective measures is desirable. Here we report the first result of mobile brain/body imaging using simultaneous high-density scalp EEG, neck and arm EMG, and body motion capture recordings from Parkinson's disease patients at onsets and offsets of bilateral subthalamic nucleus deep brain stimulation to observe resulting tremor modulation.
Materials and Methods
Parkinson's disease patients with DBS treatment (n = 3) underwent EEG recording sessions. Here we report results on one subject. EEG was recorded from 192 electrodes placed on the scalp. Electromyographic (EMG) activity was recorded from 64 surface electrodes places on the right arm surface. The subject was seated comfortably. During recording, the experimenter switched the subject's DBS unit on or off at 1-minute intervals in a 108-minute session. EEG data were analyzed using EEGLAB (sccn.ucsd.edu/eeglab). Data were low-pass filtered below 50 Hz, and were linearly decomposed using adaptive mixture independent component analysis (AMICA) to obtain time-domain independent component activities, which were converted into the time/frequency domain using a wavelet transform. Equivalent dipole models were computed for stationary scalp topographies of independent component processes originating in brain source activity.
Results
Event-related potential (ERP) analysis revealed that turning on the DBS unit produced significant EEG power suppression in the beta band (15-30 Hz) activity in bilateral sensorimotor cortices about 4-sec after DBS onset. Importantly, DBS onset was also associated with strong attenuation of EMG power near 5 Hz and its harmonics in right neck muscle source activities and right arm EMG channels. Simultaneous motion-capture data from right arm markers also showed a near 5-Hz peak.
Conclusions
This result demonstrates that DBS suppresses beta-band activity in sensorimotor cortex, as well as suppressing tremor-associated EMG activity, and establishes the feasibility of using mobile brain/body imaging (MoBI) methods to monitor modulation of brain activity associated with movement disorders.

Disclosures: J.P. Menon: None. **M. Miyakoshi:** None. **S. Lessig:** None. **S. Makeig:** None. **D. Barba:** None.

Poster

527. Parkinson's Disease: Clinical Therapies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.10/K9

Topic: C.04. Parkinson's Disease

Support: German Research Council (DFG; WE5375/1-1)

Title: Subthalamic nucleus stimulation induces cortical and corticospinal plasticity in Parkinson's disease

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Abstract: Introduction
Pathological motor processing of the subthalamo-thalamo-cortico-spinal long-range motor network is under investigation as a mechanism to explain therapeutic effects of subthalamic neuromodulation therapy in Parkinson's disease. Here, we aimed to address the modulatory effect of subthalamic nucleus deep brain stimulation (STN-DBS) on cortical activity and corticospinal long-range motor network processing.
Methods
Twenty iPD patients with STN-DBS both OFF [StimOff] and ON [StimOn] underwent externally cued finger taps simultaneous to combined EMG (flexor digitorum, FD & extensor digitorum, ED muscles) and 64-channel EEG recordings. EEG data were LaPlace transformed as spatial filter. Time-frequency decompositions of local cortical and muscular activity and long-range synchronisation (coherence) between cortex and antagonistic muscles were performed using Morlet wavelets (EEGlab / Fieldtrip). Permutation statistics using a cluster-based correction method were subsequently performed on event-related spectral pertubations to compare between treatment conditions on group-level ([StimOff] vs. [StimOn]).
Results
Significant modulations of the cortical event-related desychronisation (ERD) and of event-related corticospinal synchrony were detected: ERD in the alpha and beta bands (8-30Hz)

presented over a broad cortical area including the bilateral SM1, supplemental motor and frontal areas and was significantly facilitated with [StimOn] compared to [StimOff]. Whereas corticomuscular coherence with both FD and ED was observed mainly in the pre-movement phase similarly with both [StimOff] and [StimOn], an 'abnormal' premature rebound of beta band corticomuscular coherence with ED was observed in the [StimOff] condition (pronounced at left SM1 region).
Conclusions
Our findings demonstrate that the significant therapeutic effects of STN-DBS are paralleled by significant modulations of both cortical and corticospinal long-range motor processing.

Disclosures: D. Weiss: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Daniel Weiss is supported by a research grant from the German Research Council (DFG; WE5375-1/1). **R. Klotz:** None. **R. Govindan:** None. **M. Scholten:** None. **C. Plewnia:** None. **F. Bunjes:** None. **A. Gharabaghi:** None. **R. Krüger:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.11/K10-DP3

Topic: C.04. Parkinson's Disease

Support: NIH K23 NS067053

Title: Subthalamic deep brain stimulation synchronizes cortical activity in humans with Parkinson's disease: Intraoperative investigation of single unit discharges and scalp potentials

Authors: *H. C. WALKER, C. L. GONZALEZ, H. HUANG, B. L. GUTHRIE;
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Abstract: Introduction: Subthalamic deep brain stimulation is more effective than medications for motor symptoms of Parkinson's disease, and recent evidence suggests that alteration of activity within the cortico-subthalamic or "hyperdirect" pathway may relate to its therapeutic mechanism. Little is known about potential interactions between cerebral cortex and single unit subthalamic discharges in behaving humans with Parkinson's disease.
Methods: With prior IRB approval, we evaluated 9 subthalamic units from 4 participants with advanced Parkinson's disease who underwent surgery for routine clinical care, simultaneously performing microelectrode recordings and non-invasive scalp electroencephalography during

DBS electrode placement. We identified movement-responsive subthalamic units and compared their discharge patterns with rasters and histograms during passive versus self-paced voluntary movements of the contralateral hand. Additionally, we calculated spike-triggered averages of scalp potentials and event related potentials to the stimulus from the DBS electrode at different depths along the recording trajectory.
Results: Passive and active joint manipulation were both associated with alterations in subthalamic single unit discharge patterns. Among the units, 6/9 (67%) responded to passive finger movements, and 5/9 (56%) responded to active movements. Event related potentials to the stimulus pulse at the same location were reliably associated with polyphasic, short latency responses at the scalp with initial peak latencies as early as approximately one millisecond after the stimulus pulse. Ongoing analyses are evaluating single unit subthalamic discharges and scalp potentials and their relationship to the timing of contralateral active and passive hand movements.
Conclusions: Single unit subthalamic discharges in humans with Parkinson's disease respond readily to both passive and active contralateral hand movements, and DBS at the same site yields short latency, polyphasic ERPs that can measured non-invasively from scalp electrodes. Better characterizing the functional connectivity of the cortico-subthalamic projection has the potential to improve our understanding of the systems physiology of DBS and the pathophysiology of basal ganglia disorders.

Disclosures: H.C. Walker: None. **C.L. Gonzalez:** None. **H. Huang:** None. **B.L. Guthrie:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.12/K11

Topic: C.04. Parkinson’s Disease

Title: The effect of magnetic resonance imaging (mri) sequences on the settings and impedances of the activa pc/sc

Authors: *R. P. PATEL[1], C. S. OZA[2], S. F. DANISH[1], E. L. HARGREAVES[1];
[1]Div. of Neurosurg., Robert Wood Johnson Med. Sch., New Brunswick, NJ; [2]Biomed. Engin., Drexel Univ., Philadelphia, PA

Abstract: Deep Brain Stimulation (DBS) is an established adjunct neurosurgical treatment for movement disorders. Stimulating leads are stereotactically implanted in different targets

according to movement disorder and symptoms. Neuromodulation devices are attached to the leads and implanted subcutaneously inferior to the clavicle. Although coiled and made of a non-ferrous 80/20 platinum /iridium mix, the leads and device limit the postoperative magnetic resonance imaging (MRI) opportunities of those implanted.
Regardless, MRI during the immediate postoperative period can assist in determining proper stimulating lead placement, and detecting adverse events related to the surgery. MRI during the long term can detect lead shift, and other burgeoning brain abnormalities that may impact overall health. Thus, MRI safety is essential for effective DBS therapy management. Large MRI series have been reported for previous neuromodulation devices, but no data have been presented concerning the impact of MRI on the Activa family of devices.
Eighteen patients implanted with 21 Activa family devices (15 with 1 PC, 2 with 2 SCs, and 1 with 2 PCs) were examined during postoperative MRI sequences (ranging from a week to years post-implantation). Nine individuals underwent sequences developed in 2003 and retrospectively were determined to exceed current head coil Specific Absorption Rate (SAR) levels. The subsequent nine individuals underwent sequences that were adjusted to be more in line with the recommended SAR levels. The new SAR levels were two thirds to half those of the earlier sequences. Additionally, data were obtained from eight individuals, who underwent simple deactivations paralleling the time devices would be in the off state to undergo MRI sequences. The device activation state, the stimulation parameter set, and the monopolar and a subset of the bipolar impedances were examined.
Results indicate that neither activation state, nor stimulation parameters were altered, as a consequence of the MRI sequences, counter to what has been reported for earlier devices. Additionally, a small (<1%), but statistically significant increase in impedances was detected as a result of the MRI sequences, none of which were symptomatic. The MRI group with lessened SAR levels had less of an impedance increase then the MRI group undergoing the scans at the original SAR levels. Additionally, those with lessened SAR levels did not have impedance increases that were different than those individuals undergoing simple deactivations. Further analyses relating the impedance changes to time after implantation, baseline impedance levels, and total electrical energy delivered are underway.

Disclosures: R.P. Patel: None. **C.S. Oza:** None. **S.F. Danish:** None. **E.L. Hargreaves:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.13/K12

Topic: C.04. Parkinson’s Disease

Title: Impedance variability of the activa PC/SC across intervals of days to months during which the stimulating parameters are held constant

Authors: *N. V. PATEL[1], D. L. CAPUTO[2], R. J. DIPAOLA[2], D. MCMULLEN[1], S. F. DANISH[1], E. L. HARGREAVES[1];
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Abstract: Deep Brain Stimulation (DBS) is an established adjunct neurosurgical treatment for movement disorders. At the heart of the DBS system is the neuromodulation device, which has a number of diagnostic capabilities to ensure the integrity of the DBS system. One of these capabilities is impedance testing. We have shown previously that Medtronic's most recent Activa family (PC/SC) of neuromodulation devices are more accurate in identifying the impedance of known resistors during bench tests than their Soletra Kinetra predecessors. Recently Cheung et al., (2013) reported a number of Soletra impedance variance sources during the course of DBS therapy, including time. These sources were derived as the stimulation parameters were altered to maintain therapeutic benefit. Earlier, Sillay et al., (2010) also examined Soletra impedance variance, but did so across intervals in which the stimulating parameters were held constant, reporting no systematic change over time. Here, we replicate the Sillay et al., study for the newer Activa PC/SC neurostimulator models.
Monopolar impedances were drawn from 17 individuals at various stages of their DBS therapy (16 with single PCs, 1 with dual SCs). In total, data were collected from 32 stimulating leads, averaged across all 4 contacts. Inclusion Criteria for the impedance assessments were that the stimulating parameters be monitored for three standard therapeutic programming sessions, with the first, setting the parameters, the second keeping the parameters the same, and the third assessing the impedances prior to any programming changes. Thus, the stimulating parameters would be identical between the second and third sessions, with the impedances between these latter two sessions being of interest. The same setting interval had a mean of 64 days (3-147), while the post implantation interval had a mean of 588 days (92 to 1688). Settings were also converted to a Total Electrical Energy Delivered (TEED) measure. Results confirmed those of Sillay et al. (2010), in that there was no systematic change in impedance across the interval of same settings. The mean difference of 17.2Ohms was near identical to that of Sillay et al., although the variance was less. Unlike Sillay et al., significant relationships were found between the impedance difference and 1) the interval of same settings, as a proportion of the time from implantation (r=.37), 2) the same setting interval proportion multiplied by the TEED for the same duration (r=.50). A strong correlation between TEED and post implantation interval was also found (r=.78).

Disclosures: N.V. Patel: None. **D.L. Caputo:** None. **R.J. DiPaola:** None. **D. McMullen:** None. **S.F. Danish:** None. **E.L. Hargreaves:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.14/L1

Topic: C.04. Parkinson's Disease

Support: Michael J. Fox Foundation for Parkinson's Research, Rapid Innovation Award

Title: Intensity-dependent modulation of motor skill acquisition in Parkinson's disease by transcranial direct current stimulation

Authors: *B. J. POSTON[1], R. R. WALSH[1], E. L. HEISLER[1], J. L. ALBERTS[2];
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Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation method that has recently shown promise as a modality to increase motor function in Parkinson's disease (PD). However, the optimal stimulation parameters for impacting motor function through tDCS have not been established. The purpose was to determine the influence of the intensity of tDCS on motor skill acquisition in PD. The study was a sham-controlled, cross-over experimental design and 6 subjects diagnosed with idiopathic PD participated in the study. Subjects completed 3 experiments each separated by a 7 day washout period. Each experiment involved practice of a precision grip task (primary practice task; PPT) performed in association with 1 of 3 tDCS interventions (1 mA, 2 mA, and SHAM) in counterbalanced order. The PPT involved matching a target sine wave (0.5 Hz; target force range: 5-25% of maximum) for 10 trials that each involved matching the template for 30 s followed by a 90 s rest (total time = 20 min) while under stimulation. tDCS was applied to the scalp area overlying the hand area of the contralateral motor cortex of the primarily affected hand. SHAM stimulation was applied in the same experimental settings and according to a well-established protocol that elicits the same sensations on the surface of the scalp as application of real tDCS. Force error was quantified as the average error in force relative to the target force in the PPT. tDCS applied at 2 mA lead to better performance (12.5% lower force error) compared to SHAM stimulation. However, there was no difference in force error between the SHAM and 1 mA conditions. The findings indicate that a single application of tDCS at 2mA, but not 1 mA can increase the rate of motor skill acquisition in PD patients. Thus, tDCS may represent an intervention with a realistic potential to be translated into clinical practice because it is also relatively inexpensive, easy to operate, portable, and extremely safe. However, more research is needed to determine the optimal tDCS parameters to acutely increase motor function in PD and the extent to which chronic tDCS may further enhance these positive effects.

Disclosures: B.J. Poston: None. **R.R. Walsh:** None. **E.L. Heisler:** None. **J.L. Alberts:** None.

Poster

527. **Parkinson's Disease: Clinical Therapies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 527.15/L2

Topic: C.04. Parkinson's Disease

Title: Lack of effect of donepezil, a central cholinesterase inhibitor, on motor or cognitive performance in the MPTP macaque model of Parkinson's disease

Authors: *A. R. CROSSMAN[1], E. PIOLI[2], Q. LI[2], J. S. SCHNEIDER[3], E. BEZARD[4];
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Abstract: Central cholinesterase inhibitors have been suggested for possibly alleviating motor and non-motor symptoms in Parkinson's disease (PD) as well as L-DOPA-induced dyskinesias. A thorough examination of the effects of cholinesterase inhibitors in the gold-standard experimental models of PD is, however, lacking. The purpose of the present study was to examine the effects of donepezil, a typical cholinesterase inhibitor, on motor and cognitive performance in the MPTP-lesioned macaque models of Parkinson's disease.
The doses selected for study were 80 and 160 μg/kg (i.m.) since we had previously shown these doses to be effective in improving short-term memory in aged monkeys (assessed by the delayed matching-to-sample test on the CANTAB system), a result in line with the effects of cholinesterase inhibitors in patients with mild cognitive impairment or Alzheimer's disease. In a chronic low dose MPTP macaque model of Parkinson's disease in which animals develop cognitive deficits similar to those seen in Parkinson's disease patients, donepezil had no effect on a test of attention and working memory assessed by the variable delayed response task. Donepezil at 160 μg/kg (i.m.) also failed to improve parkinsonian motor symptoms or L-DOPA-induced dyskinesias in the gold-standard parkinsonian MPTP-lesioned macaques. As a beneficial effect on falls was reported in parkinsonian patients receiving donepezil, we investigated the potential effects of donepezil administration on posture, measuring the angles of the neck and spine. Donepezil at 160 μg/kg (i.m.) actually counteracted an L-DOPA-induced improvement in neck and spine angulation. In summary, donepezil, at the doses tested, did not improve motor or non-motor symptoms of parkinsonism, had no effect on L-DOPA-induced dyskinesias and even had a tendency to worsen posture. In conclusion, these data suggest that the supposed effect of

donepezil on improving falls in parkinsonian patients is not directly attributable to an effect on motor symptoms or the cognitive capabilities currently tested.

Disclosures: A.R. Crossman: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac Holding. **E. Pioli:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **Q. Li:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **J.S. Schneider:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac Cognition. **E. Bezard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac Holding.**Poster**

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.01/L3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: ORIP/NIH 5R240D010930-09

Title: Stereological analysis of neuronal loss in the striatum of a transgenic Huntington's disease monkey model

Authors: *Y. CHEN[1], R. VILLABA[2], S. JENKINS[2], Y. SMITH[3], A. W. S. CHAN[1]; [1]Dept. of Human Genet. and Div. of Neuropharm. and Neurologic Di, [2]Div. of Neuropharm. and Neurologic Dis., [3]Dept. of Neurology, Emory Univ. Sch. of Medicine; Div. of Neuropharm. and Ne, Yerkes Primate Res. Center, Emory Univ., Atlanta, GA

Abstract: Striatal degeneration is the key pathological feature of Huntington's disease (HD), which is caused by expansion of CAG repeats in huntingtin (*HTT*) gene. In recent years, significant effort has been devoted towards the development of a transgenic nonhuman primate model of HD. However, it is not clear whether the atrophy of the striatum in these animals is similar to that seen in HD patients. To address this issue, we assessed the extent of neuronal degeneration in the striatum of two twin brother transgenic HD monkeys that express exon 1 of human *HTT* gene with 83 and 29 CAG repeats controlled by the human ubiquitin C promoter. We euthanized one monkey, which has 83 repeats at one year old and the other at five years-old due to the severe disease phenotypes including seizure, chorea, dystonia and weight loss etc. Our recent studies have shown a positive correlation between the number of *HTT* copies and the early age of onset of motor dysfunctions (dystonia, chorea) and respiratory difficulty. First, immunohistochemistry staining with the EM48 antibody, which raised specifically against the

mutant HTT with expanded CAG repeats, revealed dense aggregation of abnormal HTT expression/intranuclear inclusion bodies throughout the brain in the two HD monkeys. Second, we estimated the volume of the putamen and caudate nucleus using the Cavalieri's method, and determined the total number of neurons in various functional regions of the striatum using the optical fractional method based on unbiased stereological principles. For this series of experiments, the caudate nucleus and putamen were divided in different regions of interest based on their functional attributes. When compared with age-matched normal monkeys, preliminary analyses indicate that both the caudate nucleus and putamen of the two transgenic animals show a decreased volume and a major neuronal loss in both striatal components. A detailed quantitative analysis is in progress to determine the extent of these changes, and compare neuronal loss across various functional regions of the striatum. Our data suggest that some of the neuropathological signatures of transgenic HD monkeys are comparable to those described in HD patients. Thus, along with our longitudinal HD development studies, transgenic HD monkeys could be a unique model for determining neuropathogenesis and identifying biomarkers of HD progression, and for the development of new HD therapies.

Disclosures: Y. Chen: None. **R. Villaba:** None. **S. Jenkins:** None. **Y. Smith:** None. **A.W.S. Chan:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.02/L4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: ARC FT3 Future Fellowship (AJH)

Title: Chronic elevation of stress hormone accelerates the onset of memory decline in Huntington's disease mice

Authors: *C. MO[1,2], T. RENOIR[1], A. J. HANNAN[1,2];
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Abstract: The onset of Huntington's disease (HD), a neurodegenerative disorder, can be predicted by the length of the causative trinucleotide repeat expansion. However, it is now known that environmental factors can also influence the onset of symptoms (cognitive, psychiatric and motor deficits). Few environmental factors have been identified. Recent data suggests that HD patients and mice show an abnormal stress response. We therefore

hypothesized that chronic treatment with corticosterone, modeling elevated stress levels, would accelerate symptom onset in HD mice.
R6/1 transgenic HD mice and wildtype littermates were treated with corticosterone dissolved in drinking water, or water alone. Treatment started from 6 weeks of age, before the onset of established cognitive, affective, sexual and motor impairments. Corticosterone (CORT) treatment did not affect sexually-induced vocalizations, affective or motor behaviours in either genotype. However, CORT-drinking HD mice developed Y-maze memory impairment earlier than water-drinking HD mice. CORT-drinking wildtype mice performed well and did not differ significantly from water-drinking controls. Hippocampal glucocorticoid and mineralcorticoid receptor mRNA, and BDNF mRNA and protein, were not altered by CORT-drinking.
The onset of memory deficits in HD mice was accelerated by CORT treatment, in contrast to wildtype littermate controls which were unaffected by the elevated CORT levels. This suggests that cognitive function in the HD brain is more vulnerable to stress-induced impairments. We present the first evidence that elevated levels of chronic stress hormone can accelerate any aspect of the HD phenotype. These findings suggest that clinical interventions which reduce stress, and associated hormone levels, may delay onset of cognitive deficits in HD individuals.

Disclosures: C. Mo: None. **T. Renoir:** None. **A.J. Hannan:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.03/L5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: AAV-mediated expression of RNAi confers long term suppression of Htt and ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease

Authors: *L. M. STANEK[1], P. S. SARDI[2], B. MASTIS[2], A. RICHARDS[2], S. H. CHENG[2], L. S. SHIHABUDDIN[2];
[1]Genzyme Corp, FRAMINGHAM, MA; [2]Neurosci., Genzyme, a Sanofi Co., Framingham, MA

Abstract: Huntington’s disease (HD) is a fatal, autosomal dominant neurogenetic disorder. HD results from a polyglutamine repeat expansion in exon 1 of the huntingtin gene, conferring a toxic gain of function on the huntingtin protein (Htt). Currently, no therapies exist that address the underlying basis of HD. RNA interference (RNAi) has emerged as a potential therapeutic strategy to treat dominant diseases by specifically reducing expression of the offending gene product. Here, we tested whether RNAi induced by artificial microRNAs (miRNAs) could

reduce expression of mutant Htt in the YAC128 transgenic mouse model of HD and improve HD-associated behavioral deficits. Recombinant AAV vectors encoding a miRNA-based hairpin against the huntingtin gene (AAV-miRNA-htt) were delivered via direct intra-striatal injections into YAC128 mice or wild type littermate controls. Both motor and affective behaviors were evaluated and tissues were harvested for histological and biochemical analysis. Animals were sacrificed 1 and 5 months post injection. Histological analysis of brain sections from injected mice showed widespread vector transduction in the striatum at 1 and 5 months post-injection without overt neuroinflammation. Biochemical analyses performed on striatal extracts showed a significant reduction in both Htt mRNA and protein levels in mice injected with AAV-miRNA-htt compared to AAV-null injected controls, with equivalent levels of reduction at both 1 and 5 months post injection. This reduction in Htt levels was associated with improvements in DARPP-32 and D1 receptor transcript levels in the striatum suggesting restoration of transcriptional dysregulation, a key molecular feature of HD. Behavioral analysis revealed that YAC128 mice injected with AAV-miRNA-htt showed significant improvements in both the Rota-rod motor coordination test and the Porsolt swim test for depression. To evaluate if lowering Htt levels could reduce neuropathology in the YAC128 mice, mice were injected with AAV-miRNA-Htt or AAV-GFP vectors at 7 months of age and sacrificed at 12 months of age, when neuropathology should be present. Immunohistochemical analysis of brain sections showed a reduction in mHtt aggregates in AAV-miRNA-Htt treated mice but not AAV-GFP injected controls. Hence, AAV-miRNA-Htt significantly reduced Htt protein expression in the brain and improved biochemical and behavioral abnormalities as well as neuropathology in the YAC128 mouse model of HD. Together, these findings confirm the potential use of AAV-mediated expression of RNAi as a therapeutic for HD.

Disclosures: L.M. Stanek: A. Employment/Salary (full or part-time):; Genzyme, A Sanofi Company. **P.S. Sardi:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **B. Mastis:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **A. Richards:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **S.H. Cheng:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **L.S. Shihabuddin:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.04/L6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: The Research Fund of IRP, NIMH, NIH

Title: Intranasal and intraperitoneal administration of a TrkB small molecule partial agonist improves motor coordination in a transgenic mouse model of Huntington's disease

Authors: *C.-T. CHIU[1], F.-F. LIAO[1], G. LINARES[1], F. M. LONGO[2], D.-M. CHUANG[1]; [1]NIMH, NIH, BETHESDA, MD; [2]Dept. of Neurol. and Neurolog. Sci., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Huntington's disease (HD) is an inherited, lethal neurodegenerative disorder currently without effective treatment. It is caused by an abnormal polyQ stretch in the protein huntingtin and results in a gradual loss of neurons particularly in the striatum and cortex. Those afflicted suffer from emotional deterioration and uncontrollable movements. Transcriptional dysregulation plays a central role in the pathology of HD and decreased expression of brain-derived neurotrophic factor (BDNF) has been reported in the striatum of HD patients. BDNF is important for cell survival and synaptic plasticity, and is a key mediator of the clinical efficacy of antidepressant drugs. However, BDNF is considered a poor drug candidate because of its relatively short half-life in plasma, weak permeability to the brain, and potential side effects as a full agonist. In this study, we assessed the therapeutic potential of LM22A-4, a small non-peptide molecule that acts as a direct and specific partial agonist of the BDNF receptor TrkB, but not p75, in a widely-used transgenic mouse model of HD, N171-82Q. Intranasal application of this compound was recently reported to produce beneficial effects in animal models of traumatic brain injury, hypoxia-ischemia, and Rett syndrome. For drug treatment, LM22A-4 was dissolved in saline and administered both intranasally (5 mg/kg) and intraperitoneally (50 mg/kg) once daily starting from seven weeks of age. Motor coordination was measured using an accelerating rotarod apparatus. Compared with age-matched wild-type littermates, the performance of N171-82Q mice at six weeks was normal but became impaired progressively and significantly at 18 (147.5 ± 6.5 vs. 124.1 ± 7.3 sec) and 22 (145.9 ± 5.5 vs. 124.3 ± 8.3 sec) weeks ($n = 12$; $P < 0.05$, t-test). This deficit in motor coordination was dramatically rescued by long-term daily treatment with LM22A-4, and the performances of drug-treated N171-82Q mice measured at 18 (150.0 ± 8.8 sec) and 22 (147.5 ± 7.5 sec; $n = 12$) weeks were comparable to those of their wild-type littermates. Since the rotarod task largely depends on striatal function, it seems possible that improved motor coordination in HD mice could be attributable to neuroprotective effects of pharmacologic activation of TrkB by this compound. Effects of LM22A-4 on depressive-like behaviors and survival of these HD mice are under investigation. We will also determine the effects of LM22A-4 on the TrkB signaling pathway, apoptosis, and huntingtin aggregates in the brain of N171-82Q mice. Our findings will provide insight into the role of TrkB as a possible therapeutic target in this disease. This study was supported by the Research Fund of IRP, NIMH, NIH.

Disclosures: C. Chiu: None. **F. Liao:** None. **G. Linares:** None. **F.M. Longo:** None. **D. Chuang:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.05/L7

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: AG031153

AG019206

NS045016

Title: Oligodendrocyte dysfunction in Huntington's disease mice

Authors: *B. HUANG, M. A. GAERTIG, X.-J. LI, S. LI;
Emory Univ., Atlanta, GA

Abstract: Huntington's disease is a neurodegenerative disorder that is caused by an expanded polyglutamine tract in the N-terminal region of huntingtin. It is characterized by cognitive and behavioral deficits, as well as movement disorders. While the effect of mutant huntingtin on neuronal function has been extensively studied, its effect on the function of glial cells remains to be fully investigated. There is evidence of white matter abnormalities in both HD patients and HD mouse models, suggesting that mutant huntingtin might affect oligodendrocyte function. We hypothesize that mutant huntingtin causes oligodendrocyte dysfunction, which contributes to the pathogenesis of the disease. To this end, we have generated transgenic mice that express an N-terminal fragment of mutant huntingtin exclusively in oligodendrocytes. These mice develop a tremor by 2 months of age and have locomotor and behavioral deficits, as well as body weight loss and reduced lifespan compared to control mice. Hypomyelination is present in the striatum of mutant mice, and preliminary data suggest that there is a dysregulation of myelin gene expression. These results suggest that the expression of mutant huntingtin in oligodendrocytes plays an important role in HD pathology.

Disclosures: B. Huang: None. **M.A. Gaertig:** None. **X. Li:** None. **S. Li:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.06/L8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI research grant

Irma Hirschl research grant

Singer foundation

Title: Characterization of the genotypes and behavioral phenotypes of mutant-huntingtin transgenic songbirds

Authors: *W.-C. LIU;
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Abstract: Huntington's Disease (HD) is a neurodegenerative genetic disorder that causes neuronal degeneration and progressive motor or cognitive dysfunction. Using lentiviral-mediated human mutant-huntingtin (mHTT, 145Q) vectors, we have recently produced transgenic songbirds (canary and zebra finch) that show severe behavioral disorders, and some of these behavioral symptoms are similar to those of human HD patients. Here we aim to characterize the genotypes and behavioral phenotypes of the transgenic songbirds that are inserted with human mutant and wild-typed huntingtin (145Q and 23Q-HTT) genes. We identify DNA, RNA, and protein expression of mutant-HTT in transgenic birds, and determine how these genotypes are associated with vocal behavioral phenotypes. Behaviorally, the F1 transgenic zebra finches show four major vocal learning disorders: (1) sustained song variability in adulthood; (2) poor song imitation; (3) progressive song deterioration; and (4) "stuttering-like" serial repetition of song syllables. The vocal disorders of 145Q F1 birds are more severe than those of wild-type 23Q F1 transgenics. These results suggest that human mutant-HTT gene is associated with progressive vocal-motor learning disorders in songbirds. The vocal learning disorders and vocal deterioration observed in transgenic songbirds and the songbird's well-defined cortical-basal ganglia vocal circuits provide songbirds a great model system to study the neuropathology that underlies HD.

Disclosure.DisclosureBlock:

Poster

528. Huntington's Disease: Animal Models II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.07/L9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Phenotypic characterization of the Q175 HD mouse model by quantitative imaging

Authors: D. SCHOLZ[1], V. MACK[1], N. BERSON[1], Y. SEDAGHAT[1], *H. VON DER KAMMER[1], C. GABRYSIAK[1], A. REICHELT[1], K. KOTTIG[1], A. EBNETH[1], I. MUNOZ-SANJUAN[2], S. KWAK[3], G. YOHRLING[4];
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder which is characterized by motor disturbance, cognitive decline and psychiatric manifestations. The disease typically starts in midlife and symptoms progress over the course of 15-20 years until death. HD is caused by a highly polymorphic CAG trinucleotide repeat expansion in the gene coding for the huntingtin protein, and aggregation of this protein, as well as gradual loss of (predominantly striatal) neurons are regarded as major hallmarks. For the investigation of disease mechanisms and potential therapeutic approaches, mouse models that recapitulate phenotypic features of human HD are crucial tools. The Q175 knock-in mouse, developed at PsychoGenics Inc. (NY, USA), contains an expanded CAG repeat (~179 CAG repeats), derived from a human mutant huntingtin allele, within the native mouse gene. In this HD model, first disease symptoms develop already at relatively young age and slowly progress over lifetime, rendering it suitable to study the course of pathological events triggered by the mutation. It has been reported that HD-like motor deficits and brain atrophy become apparent in homozygous mice by 2-3 months and in heterozygous littermates by 4-5 months of age. In order to characterize in more detail the underlying pathological processes in the heterozygous Q175 model on cellular level, we used a quantitative **immunohistochemical** approach to analyze brain sections of wild type and Q175 heterozygous mice up to 12 months of age. A series of imaging readouts were developed to detect number and morphology of particular cell types like medium spiny neurons, glial cells and oligodendrocytes in striatal and cortical regions of coronal brain sections. Furthermore, the abundance and distribution of soluble and aggregated huntingtin, labelled by antibodies against pan or mutant huntingtin, were monitored. Our detailed analyses demonstrate a progressive increase in mutant huntingtin aggregate load within the striatum and cortex of Q175 mice with age. In both brain areas, huntingtin aggregates appeared not only in the nuclear, but also in the perinuclear region of cells, with the strongest increase in aggregate number being detected between 6 and 8 months of age. However, despite the rise in aggregate load, we have neither observed degeneration of medium spiny neurons, nor any signs of inflammation as assessed on the basis of the number, morphology and size of astro- and microglia. To uncover potentially subtle changes in the CNS of the Q175 mouse model, it may be necessary to study additional biomarkers, and we are currently establishing respective imaging readouts for this purpose.

Disclosure.DisclosureBlock:The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.

Poster

528. Huntington's Disease: Animal Models II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.08/L10

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Tenovus

Title: Reversal of cognitive deficits in the Hdh^{Q111} mouse model of Huntington's disease by an α5-$GABA_A$ receptor inhibitor

Authors: R. C. MITCHELL[1], *O. F. MONTEIRO[1], J. WALLACE[1], L.-A. ETHERINGTON[1], S. SCHWEIGER[1,2], J. J. LAMBERT[1], R. F. LANGSTON[1];
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a mutation in exon 1 of the huntingtin gene resulting in an increased number of CAG repeats (>36), leading to aberrant huntingtin protein production and aggregation. HD is traditionally characterised and diagnosed by movement disturbances, however cognitive abnormalities often present prior to locomotor dysfunction and may occur, in part, through changes within the hippocampus [1]. We assessed cognitive abnormalities in the Hdh^{Q111} mouse model of HD in which exon 1 of the huntingtin gene contains 111 CAG repeats. Cognition was assessed in two age groups (1.5 and 3 month old mice) using a suite of novel object recognition tasks in wild type (WT) and heterozygous (HET) mice. Both young (1.5 month) WT and HET mice performed all the tasks and their behaviour was indistinguishable. For older WT mice (3 months) performance was maintained on all tasks, but HET mice now performed at chance levels on tests of hippocampal-dependent memory. Furthermore, hippocampal CA1 long term potentiation was impaired in HET mice *cf.* WT. The hippocampus exhibits dense expression of α5-$GABA_A$Rs [2] and drugs inhibiting this $GABA_A$R subtype are known to enhance cognition [3]. 3 month old WT and HET mice were treated with the α5-$GABA_A$R inverse agonist α5IA, or vehicle. Although the drug had no effect on the performance of WT mice, it successfully alleviated all the cognitive deficits exhibited by HET mice. These data identify the α5-$GABA_A$R as a viable target for treating the cognitive symptoms of HD.

[1] Lynch *et. al.* (2007). J. Neurosci. [2] Caraiscos *et al.*,(2004) PNAS [3] Dawson *et al.* (2006), J. Pharmacol. Expt. Ther.
This work was funded by Tenovus

Disclosures: R.C. Mitchell: None. **O.F. Monteiro:** None. **J. Wallace:** None. **L. Etherington:** None. **S. Schweiger:** None. **J.J. Lambert:** None. **R.F. Langston:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.09/L11

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Canadian Institutes of Health Research (CIHR)

Huntington Society of Canada (HSC)

Alberta Innovates Health Solutions (AIHS)

Title: Ganglioside GM1 ameliorates non-motor symptoms in the YAC128 model of Huntington disease

Authors: M. ALPAUGH[1,2], P. KAR[1], D. GALLEGUILLOS[1,2], M. HORKEY[1], B. KERR[2,3], *S. SIPIONE[1,2];
[1]Dept. of Pharmacol., [2]Ctr. for Neurosci., [3]Dept. of Anesthesiol. and Pain Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Huntington disease (HD) is a neurodegenerative disorder that results in motor, cognitive and psychiatric deficits. The disease is caused by the expansion of a polyglutamine stretch in huntingtin, a ubiquitous protein with unclear functions.
The molecular mechanisms underlying neurodegeneration in HD are complex and include transcriptional dysregulation, mitochondrial dysfunction, impaired intracellular and axonal transport, as well as aberrant signaling and neurotransmission.
We recently demonstrated that the synthesis of ganglioside GM1, a lipid highly enriched in the brain, is also impaired in a variety of HD models. Restoration of normal GM1 levels by chronic intra-ventricular infusion of the ganglioside, abolished the pathological motor phenotype in already symptomatic YAC128 mice, a well characterized transgenic model of HD. These dramatic effects on mouse motor behaviour were accompanied by phosphorylation of mutant

huntingtin at Ser13 and Ser16, a post-translational modification that has been shown to decrease mutant huntingtin toxicity. This suggests that GM1 might be able to modify the course of HD and also correct non-motor symptoms of the disease.
Our preliminary data support this hypothesis. In the YAC128 model, significant attenuation of cognitive and psychiatric symptoms associated with HD was observed after administration of GM1 for 14 days. YAC128 mice treated with GM1, but not with vehicle, performed as well as wild-type mice in tests that measure anxiety (elevated plus maze, open field, and novelty induced hypophagia), cognition (y-maze, novel mouse approach), and depression (forced swim test). Overall, our data suggest that GM1 is a disease-modifying treatment that is able to ameliorate not only motor dysfunction, but also cognitive and psychiatric deficits in YAC128 mice.

Disclosures: M. Alpaugh: None. **P. Kar:** None. **D. Galleguillos:** None. **M. Horkey:** None. **B. Kerr:** None. **S. Sipione:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.10/L12

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Impaired thermoregulatory capacity in R6/2 and zQ175 mouse models of huntington's disease during cold challenge

Authors: J. PUOLIVÄLI[1], E. HOHTOLA[2], *O. M. KONTKANEN[1], L. C. PARK[3], T. HEIKKINEN[1];
[1]Charles River Discovery Res. Services, Kuopio, Finland; [2]Dept. of Biol., Univ. of Oulu, Oulu, Finland; [3]CHDI Fndn. Inc, Los Angeles, CA

Abstract: Huntington's disease (HD) patients display clinical signs of disturbed energy metabolism, and similar changes have been observed in transgenic HD mouse models. Negative energy balance in HD may cause body weight decrease and loss of muscle bulk. Here we studied the energetic profile of two different HD mouse models, transgenic R6/2 and zQ175 knock in (KI) HOMO and HET mice, as well as WT control mice. Resting oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured by indirect calorimetry at thermoneutral zone (+32°C) and during cold stress (slow cooling from 32 to +ca. 3°C). The basal metabolic rate (BMR) at thermoneutrality was measured and compared to maximal energy use during the cold challenge. In addition, the body temperature (Tb) was measured with a rectal probe during challenge (at +32°C, +20°C, and +3°C). RQ (respiratory quotient, CO_2 produced/O_2 consumed)

and metabolic scope (VO_2 Max/BMR) values were also calculated. R6/2 mice were measured at 12 weeks old, and zQ175 KI mice at 12 months old. R6/2 and HOMO zQ175 KI mice showed increased mass-specific BMR at thermoneutrality compared to WT mice. In addition, R6/2 mice also showed decreased mass-specific maximum energy expenditure under cold challenge, and both R6/2 and KI HOMO mice showed decreased metabolic scope. However, KI HET mice had BMR and metabolic scope values similar to WT mice. We also found that R6/2 mice had extremely decreased Tb values during cold challenge; whereas, the Tb in KI mice maintained similar to WT mice. The absolute energy expenditure over the whole temperature range (+32 to +3°C) was linearly correlated in WT and KI mice, but not in R6/2, suggesting the thermogenic capacity in R6/2 is compromised. In summary, aged R6/2 and zQ175 KI HOMO mice both display increased metabolic rate at thermoneutrality, and R6/2 mice also lower maximum metabolic rate during cold stress. Thermogenic capacity in HD with energetic deficiency may be a useful screening tool for potential therapeutic compounds, particularly those that potentiate energy metabolism.

Disclosures: J. Puoliväli: None. **E. Hohtola:** None. **O.M. Kontkanen:** None. **L.C. Park:** None. **T. Heikkinen:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.11/L13

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Immunohistochemical characterization of R6/2 transgenic and zQ175 knock in mouse models of Huntington’s Disease

Authors: *M. CERRADA-GIMENEZ[1], L. TÄHTIVAARA[1], L. C. PARK[2], D. HOWLAND[2], I. MUÑOZ-SANJUAN[2], O. KONTKANEN[1], N. VARTIAINEN[1];
[1]Charles River Discovery Res. Services Finland Ltd, Kuopio, Finland; [2]CHDI Fndn. Inc., Los Angeles, CA

Abstract: Huntington's disease (HD) is accompanied by several tissue, cell type and pathway dysfunctions. Increased inflammatory response, oxidative and nitrosative stress, as well as altered iron metabolism are associated with the disease progression. We have immunohistochemically and histologically characterized changes in neuroinflammatory response in the brain of two HD mouse lines: widely-used R6/2 transgenic and a recently introduced zQ175 knock in (KI) mice (Heikkinen et al., *Plos One* 2012:7(12):e50717; Menalled et al., *Plos*

One 2012:7(12):e49838). The disease phenotype in the R6/2 mouse is early-onset and rapidly progressing, while the zQ175KI mouse exhibits a late-onset of symptoms that more closely mimic those in HD patients. zQ175 KI mice show age and genotype-related cognitive deficit as well as striatal atrophy at 8-10 months of age, so we wanted to evaluate the immunohistochemical markers at the same age range. R6/2 and zQ175KI mouse brain tissues were collected at different
ages: 4, 8 and 12 weeks for R6/2 and 3, 6 and 9 months for zQ175 KI mice. For basic characterization the neuronal marker NeuN and the astroglial marker GFAP were used. Further characterization involved immunohistochemistry for HD-associated markers like DARPP-32 (dopaminergic neurons), EM-48 (huntingtin protein), reactive nitrogen species-related markers nitrotyrosine, as well as inflammatory cell (microglial) marker Iba-1. Furthermore, immunohistochemical analysis for the iron storage protein ferritin and histological Perls staining for iron were used. The different immunostainings were analyzed for number of immunoreactive cells (NeuN, GFAP, Iba-1), size of immunopositive cells (Iba-1), staining intensity or amount of immunoreactivity (DARPP-32). In R6/2 mice, stereological analysis of Iba-1 revealed increased soma size, a clear sign of activated microglia, when compared to wild-type littermates. The immunoreactivity of ferritin was significantly increased in the R6/2 mice, and the cell morphology was similar to activated microglia. The immunoreactivity of DARPP-32 in striatum was significantly decreased in R6/2 mice when compared to wild-type mice. Our study provides new information on the inflammatory and oxidative damage during disease progression in two HD mouse models. Several of the immunohistochemical markers used may be useful as biomarkers when evaluating disease progression and compound efficacy in preclinical models.

Disclosures: M. Cerrada-Gimenez: None. **L. Tähtivaara:** None. **L.C. Park:** None. **D. Howland:** None. **I. Muñoz-Sanjuan:** None. **O. Kontkanen:** None. **N. Vartiainen:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.12/L14

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: RR018827

RR00165

Title: Progressive motor impairment in transgenic Huntington disease monkeys

Authors: J. BACHEVALIER, T. CHI, E. HEIDI, S. MORAN, *A. W. CHAN;
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the expansion of polyglutamine (CAG; Q) repeat within the coding region of the Huntingtin (*HTT*) gene. HD is a devastating disorder with progressive decline in motor, cognitive and psychiatric functions. This paper reports a longitudinal study of motor functions in a transgenic HD nonhuman primate (HD-NHP). rHD1, a HD-NHP expressing exon 1 of human *HTT* gene with 29Q regulated by a human ubiquitin C promoter. rHD6, 7 and 8 express Exon 1-10 of the *HTT* gene containing 73Q under the control of human *HTT* promoter. Additionally, four age-matched control NHPs were used for comparisons. All animals were tested at different points during development to follow their neurobehavioral and motor development. Except for the INAS and JNAS task that were performed in the infant nursery, all other tasks were conducted in a sound-attenuated room equipped with a white noise generator to reduce external noise, except otherwise mentioned. Animals were transferred to a Wisconsin General Testing Apparatus, facing a test tray onto which objects or equipment could be positioned. Rewards were either peanuts, raisins, fruity gems, mini M&M, Marshmallows, depending on animal's preference, or a ring-shaped candy ("Lifesaver") for the ***Visuospatial orientation*** task at 16 and 36 months. The result showed that rHD1 had neuromotor responses and motor activities similar to those of controls, the rest other three HD infant displayed slightly reduced neuromotor responses in Week 3 and 5 ($U = 2$, $p < .04$ and $U = 1$, $p < .04$) and poorer motor control at all weeks (all $p_s < .03$ for Weeks 2-5). The neuromotor responses of the 3 HD-NHPs improved slowly with age but never reached the strength of those of the control animals [Group effect: $F(1, 6) = 12.46$, $p < .01$]. At 16 months, rHD1 showed no impairment in the Detour/Barrier task, whereas more problems emerged in the other 3 HD-NHPs. They displayed significantly greater motor problems when attempting to retrieve the food rewards in all trials [$U = 0.5$, $p = .04$ and $U = 0$, $p = .026$, for easy and difficult trials, respectively]. The latency and the fastest time to free the lifesaver and the number of failure at 16 months did not differ between groups, with all four HD-NHPs performing as well as the control animals. By 36 months of age, however, HD-NHPs required longer time to free the candy when more difficult patterns were used [$t(5) = 3.73$, $p = .014$, for latency]. Our study shows a consistent progression of motor impairments in HD-NHPs. The data provide the first primate model that replicates progression of HD in human based on longitudinal behavioral measurements. Grant supports: RR-00165 and RR-018827.

Disclosures: J. Bachevalier: None. **A.W. Chan:** None. **T. Chi:** None. **E. Heidi:** None. **S. Moran:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.13/L15

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Functional imaging in awake mice: Studies on a transgenic (Q175) mouse model of Huntington's disease

Authors: *C. F. FERRIS[1], T. M. BARCHET[1], S. TODDES[2], P. KULKARNI[1], J. YEE[1], W. KENKEL[1], M. NEDELMAN[3];
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Abstract: Functional magnetic resonance imaging (fMRI) is a powerful and widely-used tool for investigating changes in brain function and structure in humans. Functional fMRI is also becoming more commonly used to study the neurobiology of the brain in awake rats and non-human primates. However, there are very few, if any, published reports on the functional connectivity, tractography, quantitative anisotropy or functional brain imaging in awake mice. To address this need, we developed a combined radiofrequency coil and mouse restraining system for awake imaging, together with a 3D segmented and annotated mouse atlas and software for computational data analysis of over 100 discrete brain areas for C57BL mice. This technology was applied to the characterization of the transgenic Q175 mouse model of Huntington's disease imaged at 7.0T (300 MHz). Wild type, heterozygous and homozygous Q175 mice were acclimated to the restrainer and imaging protocol before a scanning session. The stability of the data (motion detection) as estimated by a 3D rigid body model with six degrees of freedom for translational and rotational movement was determined for all animals (n=28) and was less than 20 μm and 1.5-3 degrees. Blood Oxygen Level Dependent (BOLD) data was obtained with a multi-slice, single-shot Fast Spin Echo pulse sequence using a partial Fourier acquisition with a 9/16 ratio. Twenty, 0.75 mm thick, axial slices were collected every six seconds. With a FOV of 2.5 cm and a data matrix of 96 x 96, the in-plane pixel resolution for these studies was 260 μm2. A surrogate BOLD response was elicited by the presentation of 5% CO2 and revealed robust signal changes (>6%) in all animals. Animals challenged with the odor of ferret to elicit the innate fear response and the odor of almond to activate neural circuitry involved in motivation and reward (Kulkarni et al. Behav. Brain Res, 230:201, 2012) show significant increases and decreases in BOLD signal in brain areas involved in emotional experience and cognition. Diffusion tensor imaging (DTI) with quantitative anisotropy was performed to detect differences in brain microarchitecture between the different groups. To do so, we used a novel method of analysis in which different indices of anisotropy (IA), e.g. ADC, FA, λ1, RA from over 20,000 voxels were registered into the mouse atlas. There were significant differences in IA values in multiple areas of the brain between the wild-type controls and homozygous Q175. These data support the use of non-invasive MRI in awake mice and open up

the possibility for assessing the efficacy of new therapeutics on the myriad of transgenic mouse models for psychiatric and neurological disorders.

Disclosures: C.F. Ferris: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging, Animal Imaging Research. **T.M. Barchet:** None. **J. Yee:** None. **W. Kenkel:** None. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **S. Toddes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Animal Imaging Research. **M. Nedelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.14/L16

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Fellowship-Conacyt

Fellowship-PIFI

Title: Fatty acid enriched diet confers neuroprotection in a Huntington´s disease model: Studies *In vitro* and in silico

Authors: *A. MORALES[1,2], A. SANCHEZ[3], D. GONZALEZ[5], S. MONTES[5], M. EL HAFIDI BENTLAKDER[4], E. SORIA[3], C. RÍOS[5], A. ZAMORANO[6], F. PÉREZ[5];
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Abstract: Essential fatty acids (EFAs) exert experimental and clinical neuroprotection in neurodegenerative diseases as Huntington's disease (HD). The main EFAs; oleic acid (OA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contained in olive and fish oil, are inserted into the cell membranes and they act as agonists of nuclear receptors. Althought that EFAs confer neuroprotection, the exact mechanism is still unknown. The aim of this work was to

evaluate the neuroprotection given by a diet rich in EFAs, by means of in vitro (synaptosomes) and in silico models, evaluating the membrane insertion of EFAs and the induction of nuclear signals. Male Wistar rats (140-150 g) were fed during 20 days with control diet or EFAs enriched diet contained in olive or fish oil (15% w/w). Synaptosomes viability was evaluated by electron microscopy and measured gamma-glutamyl transpeptidase activity was measured. Synaptosomes lipid profile showed a 21.9% OA enrichment for the group of olive oil and 0.12% of EPA in fish oil group compared to the control diet. Membrane fluidity evaluation, showed reduced anisotropy in enriched synaptosomes. Synaptosomes incubation with quinolinic acid (QUIN, an agonist NMDA receptor), showed significant oxidative reduction in enriched EFAs group compared with control. Finally significative expression of gamma isoform of peroxisome proliferator activated receptors (PPARy) was detected in EFAs-enriched striata tissue vs control. Molecular dynamics simulations were performed in parallel, inserting DHA and EPA systems in a reduced membrane model (DPPC). These results suggest that EFAs enrichment confers antioxidant effect, involving membrane integrity and induction of PPARy.

Disclosures: A. Morales: None. **A. Sanchez:** Other; National Institute of Cardiology - Ignacio Chávez. **D. Gonzalez:** Other; THE NATIONAL INSTITUTE OF NEUROLOGY AND NEUROSURGERY. **S. Montes:** Other; THE NATIONAL INSTITUTE OF NEUROLOGY AND NEUROSURGERY. **M. El Hafidi Bentlakder:** Other; The National Institute of Cardiology - Ignacio Chávez. **E. Soria:** Other; National Institute of Cardiology - Ignacio Chávez. **C. Ríos:** None. **A. Zamorano:** Other; IPN. **F. Pérez:** Other; NATIONAL INSTITUTE OF NEUROLOGY AND NEUROSURGERY.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.15/L17

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation BACHD rats

Title: Early abnormal striatal gene expression in BACHD rats recapitulates the human disorder and reveals multiple mechanisms of gene dysregulation in Huntington Disease

Authors: *L. YU-TAEGER, M. BONIN, O. RIESS, H. P. NGUYEN;
Inst. of Med. Genet. and Applied Genomics, Univ. of Tuebingen, Tübingen, Germany

Abstract: Huntington Disease (HD) is an autosomal dominant inherited neurodegenerative disorder caused by an expansion of CAG repeats in the HTT gene. The mutant huntingtin protein

(htt) has been proposed to cause neuronal dysfunction and neuronal loss through multiple mechanisms. Transcriptional changes may be a core pathogenic feature of HD. Utilizing the Affymetrix platform we performed a genome-wide RNA expression analysis in two BACHD transgenic rat lines at 12 months of age, which carry full-length human mutant huntingtin with different expression levels. Microarray results were validated through real-time PCR with another cohort of rats, showing up- and downregulation in numerous striatal genes in BACHD rats compared to wild type controls. Comparison with data from human HD brains revealed a high concordance in distinct canonical pathways and functional categories, while the analysis of upstream regulators identified these abnormalities to be involved in multiple mechanisms including cytokine signaling and growth factor signaling. Results of this study demonstrate that this BACHD rat model recapitulates accurately gene expression changes of the human disease and therefore supports its application in preclinical research. Additionally, our results point to previously unknown mechanisms in gene dysregulation and may provide novel insights into HD pathogenesis.

Disclosures: L. Yu-Taeger: A. Employment/Salary (full or part-time):; CHDI Foundation. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CHDI Foundation. **M. Bonin:** None. **O. Riess:** None. **H.P. Nguyen:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CHDI Foundation.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.16/L18

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: HD-SELECT, a novel patient-derived cell model enabling disease-modifying drug selection for Huntington’s disease

Authors: C. BISCARRAT[1], *N. COMPAGNONE[2];
[1]Translational medicine, [2]ICDD, MEYREUIL, France

Abstract: Huntington’s disease (HD) is an autosomal dominant monogenetic disease that alters muscle coordination and leads to striatal neurodegeneration and dementia. HD may begin with uncontrolled movements in the fingers, feet, face or trunk, but it also affects the patient’s judgment, memory and other cognitive functions. HD progression results in slow degeneration in

neurons controlling emotions, cognitive functions and coordination of movements, leading to death of patients, mostly due to malnutrition, in an average of 16 years.
Lack of translatable models for complex neurodegenerative diseases has contributed to drug development failure in HD. Animal models fail to recapitulate the disease progression and complexity as seen in humans, while *in vitro* models, generally focused on a molecular target, only partially mimic the disease etiology. However, over the last decade, both have largely increased our understanding of HD pathophysiological mechanisms, as well as the number of drug development programs targeting HD. Finding a cure for HD is crucial, and the biotech and pharma industries lack proper models to guide the selection of disease-modifying drugs. To this aim, ICDD identifies and develops patient-derived cell models that enable the exploration of disease-modifying property from small molecules or biologics. Using a system biology approach and a proprietary cellulomic technology, Mitostream®, a HD signature was identified from a pilot cohort of 12 patients. The cohort included Caucasian female patients 38.17 +/- 12.6 years of age with an average duration of the disease at the time of biopsy of 3.7 years +/-10.3 years. Age-matched controls ranged 36.33 +/- 14.4 years of age. No other diseases or co-morbidity were reported in the HD group, while a minor depression was reported in the healthy group. Data reduction and identification of the discriminating variables were obtained through the use of different complementary strategies using principal component analysis, hierarchical cluster classification and discriminant analysis. It generated a HD specific disease-signature of 7 variables that defined the group of HD patients and that could be partially reversed with reference compounds. The signature specificity was tested in a larger population of 32 patients including patients with other neurodegenerative diseases and proved specific for HD. The model was stable and the signature robust over time, making the developed HD-select model a good tool for disease-modifying compound selection.

Disclosures: C. Biscarrat: None. **N. Compagnone:** None.

Poster

528. **Huntington's Disease: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 528.17/M1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Genetic knockdown of HDAC4, or sub-chronic treatment with a novel selective Class IIa HDAC inhibitor, reverses elevated membrane excitability in striatal medium spiny neurons from R6/2 and zQ175 Huntington’s disease model mice

Authors: *G. C. TOMBAUGH[1], S. GELMAN[1], A. BRADAIA[2], K. WADEL[2], V. GARDES[2], C. TOULLER[2], A. SERS[2], A. GHAVAMI[1], B. BUISSON[2], G. BATES[3], M. MIELCAREK[3], C. DOMINGUEZ[4], M. MAILLARD[4], V. BEAUMONT[4];
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Abstract: Huntington's disease (HD) is a lethal autosomal dominant neurodegenerative disorder that leads to deficits in motor control widely believed to reflect structural and/or functional changes in neurons of the basal ganglia. In-vitro brain slice recordings of both the R6/2 and zQ175 mouse models of HD have revealed changes in intrinsic membrane properties of striatal medium spiny neurons (MSNs), which are selectively vulnerable in HD. Among these changes is a large increase in membrane resistance (Rm) and a reduced rheobasic current (Rh), defined as the minimum depolarizing current needed to elicit an action potential. Such changes may result in aberrant processing of excitatory input to the striatum. Genetic-knock down of HDAC4 restored these and other electrophysiological changes in both the R6/2 model, a transgenic over-expresser of Exon 1 HTT with an expanded polyglutamine repeat, and heterozygous zQ175 knock-in mice, which carry one normal and one mutant HTT allele with an expanded repeat of ~190 polyglutamines, in addition to reversing behavioral alterations in R6/2 mice.
We examined MSN properties in R6/2 mice and zQ175 heterozygous knock-in mice after sub-chronic in vivo exposure (4 weeks or 4 months respectively) to a novel selective Class IIa HDAC inhibitor, CHDI-00390576 (up to 100mg/kg, p.o., bid) to evaluate whether this could mimic the genetic HDAC4 knockdown data. Whole-cell recordings from visually identified MSNs were performed in slices from 8w old R6/2 mice and 6mo old Q175 mice and respective WT littermates, 1day - 2 weeks following drug washout. MSNs in R6/2, Q175 and WT mice had nearly identical resting membrane potentials, but R6/2 and Q175 MSNs exhibited significantly elevated Rm and lower Rh. Rm in both models was partially reversed by CHDI-00390576; Rh was partially reversed in R62 but not significantly in Q175. Neither Rm nor Rh was affected in drug-treated WT mice. Action potential amplitude and threshold in the HD models were decreased and increased, respectively, relative to WT controls. These changes, which were reversed in HDAC4 knockdown Q175 mice, were unaffected in drug-treated R62 mice. In R62 input-output curves for EPSCs evoked by cortical stimulation (layer V-VI) saturated at a lower amplitude, and mEPSC frequency was significantly lower compared to WT MSNs, but no drug-related rescue was seen for either measure. In contrast, reduced mEPSC frequency was reversed by HDAC4 knockdown in both R62 and Q175. These findings indicate that disease-specific HD phenotypes in MSNs can be partially reversed by manipulating HDAC4 activity/expression in R6/2 and Q175 mice, suggesting a therapeutic potential for Class IIa HDAC inhibitors in HD.

Disclosure.DisclosureBlock:The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.01/M2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: The expression of mutant SOD1 in astrocytes negated the noradrenaline-induced astrocyte-mediated neuroprotection

Authors: *Y. YOSHIOKA, M. AKUNE, T. YOSHIDA, A. YAMAMURO, Y. ISHIMARU, S. MAEDA;
Setsunan Univ., Hirakata, Japan

Abstract: It has been reported that dysfunction of astrocyte might be one of the cause of motor neuron degeneration in the transgenic SOD1 (G93A) mouse model of amyotrophic lateral sclerosis (ALS). The reduction
of glutathione (GSH) has been also reported in the spinal cord of mutant G93A-SOD1 transgenic mice. Recently, we found that noradrenaline (NA) protected neurons from H_2O_2-induced death by increasing the supply of GSH from astrocytes by using the co-culture system of human neuroblastoma SH-SY5Y cells and human astrocytoma U-251 MG cells. We hypothesize that NA-induced astrocyte-mediated neuroprotection might be impaired in familial ALS. To reveal the hypothesis, in this study, we investigated the effect of mutant SOD1 (G93A) expression in U-251 MG cells on the neuroprotective effect of NA by using the co-culture system. We established U-251 MG cells overexpressed wild-type or mutant (G93A) human SOD1 and SH-SY5Y cells overexpressed green fluorescence protein (GFP). The viability of SH-SY5Y cells was determined based on the morphology of GFP-positive cells under a
fluorescence microscope. To investigate the changes of intracellular GSH level in SH-SY5Y cells in co-culture, the cells were stained with reduced GSH-reactive probe monochlorobimane, and were analyzed by Cellomics ArrayScan. NA (10 μM) increased intracellular GSH levels in U-251 MG cells expressing wild-type SOD1, but not in the cells expressing mutant SOD1. In co-culture of SH-SY5Y cells and U-251 MG cells expressing wild-type SOD1, NA (10 μM) increased intracellular GSH levels of SH-SY5Y cells and protected the cells from H_2O_2-induced death. On the other hand, in co-culture using U-251 MG cells expressing mutant SOD1, NA (10 μM) neither affected intracellular GSH levels of SH-SY5Y cells nor protected the cells from H_2O_2-induced death. These results suggest that mutant SOD1 negated NA-induced astrocyte-mediated neuroprotection by inhibiting the increase of GSH in astrocytes. These findings indicate that dysfunction of the neuroprotective system induced by NA might be one of the cause of motor neuron degeneration in familial ALS.

Disclosures: Y. Yoshioka: None. **M. Akune:** None. **T. Yoshida:** None. **A. Yamamuro:** None. **Y. Ishimaru:** None. **S. Maeda:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.02/M3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Fund for Scientific Research Flanders

ALS Association

Research Fund KULeuven

IWT

Title: Deleting ephrin-b2 from reactive astrocytes is beneficial in ALS

Authors: L. SCHOONAERT[1,2], L. POPPE[1,2], A. VAN HOECKE[3], *W. L. ROBBERECHT[4,1,2]; [1]Lab. of Neurobiology, Vesalius Res. Center, VIB, Leuven, Belgium; [2]Exptl. Neurol. (Department of Neurosciences) and Leuven Res. Inst. for Neurosci. and Dis. (LIND), Univ. of Leuven (KU Leuven), Leuven, Belgium; [3]Max-Planck-Institute of Neurobio., München, Germany; [4]Dept Neurol., Univ. Hosp Gasthuisberg, 3000 Leuven, Belgium

Abstract:
Recently it has been shown that the EphA4 receptor is a modifier of ALS. Genetic and pharmacological inhibition of EphA4 rescues the phenotype in the zebrafish model of ALS and increases survival in ALS rodent models. In ALS patients an inverse correlation was found between EphA4 expression and disease onset. However, it is not yet fully elicidated what the mechanism of action is. Remarkably it is known that EphA4 interacts both with ephrin-a and ephrin-b ligands, wich are also bound to the cell membrane by a GPI-anchor or a transmembrane domain respectively. Several of the EphA4 interaction partners have been shown to be expressed on reactive astrocytes, microglia and oligodendrocytes. These cells play an important role in the pathogenesis of ALS and surround motor neurons which abundantly express EphA4. A promising candidate was ephrin-b2 as it has been shown to be highly expressed by reactive astrocytes after spinal cord injury. In the spinal cord of WT SOD1 mice ephrin-b2 was highly

expressed in motor neurons while only faint expression could be detected in astrocytes. At symptomatic stages the expression pattern changes and high immunoreactivity could be detected in astrocytes while the neuronal expression diminished. Similar results were obtained in spinal cords from ALS patients and controls. Although the expression pattern of ephrin-b2 changes during disease progression, the overall expression stays the same as checked by RT-PCR analysis. We hypothised that deleting ephrin-b2 from reactive astrocytes might have a beneficial effect on ALS. For this purpose we crossed the conditional ephrin-b2 knockout mouse with a GFAP-specific Cre-line and the $SOD1^{G93A}$ ALS model. Even though the GFAP-Cre promoter shows leaky expression, we find delayed disease onset and prolonged disease duration. These results suggest that ephrin-b2 might play a role in modifying Amyotrophic Lateral Sclerosis, but it will need further investigation.

Disclosures: L. Schoonaert: None. **L. Poppe:** None. **A. Van Hoecke:** None. **W.L. Robberecht:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.03/M4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Grant-in-Aid for Scientific Research on Innovative Areas from The Ministry of Education, Culture, Sports, Science and Technology (MEXT)

Title: Calpain-dependent cleavage of TDP-43 plays a crucial role in ALS pathology

Authors: *T. YAMASHITA[1,3,2], T. HIDEYAMA[2], S. TERAMOTO[1,3,2], J. TAKANO[4], N. IWATA[5], T. C. SAIDO[4], S. KWAK[1,2,6];
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Abstract:
TAR DNA-binding protein (TDP-43) pathology is the pathological hallmark of sporadic ALS,

but the underlying mechanism is poorly understood. Notably, TDP-43 pathology and reduced expression of adenosine deaminase acting on RNA 2 (ADAR2), the RNA editing enzyme responsible for adenosine-to-inosine conversion at the GluA2 glutamine/arginine (Q/R) site, concomitantly occur in the same motor neurons in ALS patients, suggesting a link between these two ALS-specific molecular abnormalities. AMPA receptors containing Q/R site-unedited GluA2 in their subunit assembly are Ca^{2+}-permeable, and motor neurons lacking ADAR2 undergo slow death, suggesting a role of Ca^{2+}-permeable AMPA receptor-mediated neuronal death mechanism in the ALS pathogenesis.
We found that Ca^{2+}-dependent serine protease calpain cleaved TDP-43 at the C-terminal region, and resulting N-terminal fragments were aggregation-prone. Notably, TDP-43 was absent from the nucleus forming aggregates in the cytoplasm of the motor neurons in a manner dependent on calpain activation in conditional ADAR2 knockout (AR2) mice, a mechanistic ALS model in which the ADAR2 gene is targeted in cholinergic neurons including motor neurons. Abnormal processing of TDP-43 was inhibited when the Ca^{2+}-impermeable AMPA receptors was expressed or when calpastatin, the endogenous calpain inhibitor, was overexpressed, but conversely, TDP-43 mislocalization was exaggerated when calpastatin was knocked out. Calpain cleaved TDP-43 at several positions in the C-terminal region, and calpain-dependent TDP-43 fragments were demonstrated in the brains and spinal cords of ALS patients. Taken together, the calpain-dependent cleavage of TDP-43 plays a crucial role in ALS pathology.

Disclosures: T. Yamashita: None. **T. Hideyama:** None. **S. Teramoto:** None. **J. Takano:** None. **N. Iwata:** None. **T.C. Saido:** None. **S. Kwak:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.04/M5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

the CREST/JST

Title: FUS-regulated region- and cell-type-specific transcriptome is associated with cell selectivity in ALS/FTLD

Authors: *Y. FUJIOKA[1], S. ISHIGAKI[2], A. MASUDA[3], Y. IGUCHI[2], T. UDAGAWA[2], H. WATANABE[2], M. KATSUNO[2], K. OHNO[3], G. SOBUE[2];

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Abstract: Amyotrophic
lateral sclerosis (ALS) is characterized by selective motor neuron degeneration in the primary motor cortex, brainstem and spinal cord. FUS is the causative gene of familial ALS and pathologically linked to sporadic ALS and frontotemporal lobar degeneration (FTLD). FUS is an RNA binding protein which was reported to function in transcription, RNA splicing, and RNA transport. Accumulated lines of evidence have suggested that altered RNA metabolism would be in the center of ALS/FTLD pathogenesis. To clarify the RNA metabolism cascade regulated by FUS in ALS/FTLD, we compared the FUS-regulated profiles of gene expression and alternative splicing in different primary cells from the central nervous system. FUS differentially regulated more genes in motor neurons, cortical neurons, and glial cells than in cerebellar neurons. The profiles of FUS-mediated alternative splicing in cortical neurons were quite similar to those in motor neurons, but not in glial cells or cerebellar neurons, whereas FUS-mediated gene expression profiles were similar among cortical neurons, motor neurons, and glial cells. FUS-mediated regulation of alternative splicing and gene expression likely determines vulnerability of cells and cellular response, respectively. We found that the Fus-binding-positions on target splicing exons were similar between glial cells and cortical neurons. Certain neurological diseases-associated genes, including *Mapt, Stx1a,* and *Fmr1,* were regulated by FUS. Our results indicate that FUS-regulated transcriptome profiles seem relevant to cell vulnerability in FUS-associated ALS/FTLD. Identified RNA targets for FUS could be therapeutic targets for ALS/FTLD.

Disclosures: Y. Fujioka: None. **S. Ishigaki:** None. **Y. Iguchi:** None. **T. Udagawa:** None. **H. Watanabe:** None. **M. Katsuno:** None. **G. Sobue:** None. **A. Masuda:** None. **K. Ohno:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.05/M6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Muscular Dystrophy Association

Title: Modification of disease onset and progression for ALS by human chromogranin B variants

Authors: *Y. OHTA, D. PHANEUF, J.-P. JULIEN;
Psychiatry and Neurosci., Ctr. de recherce du CHU, Laval Univ., Quebec, QC, Canada

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disease that is characterized by selective loss of central and peripheral motor neurons. Although most cases of ALS are sporadic (sALS), approximately 15-20% of familial ALS (fALS) cases hold a variety of genetic mutations in the Cu/Zn superoxide dismutase (SOD1) gene. The mechanism whereby mutant SOD1 causes specific degeneration of motor neurons remains unclear, but it is believed that disease is due to a gain of a toxic function of mutant SOD1 protein. Recently, we discovered that chromogranin A and B interact specifically with different mutant forms of SOD1 (Urushitani M, et al. 2006) and that ALS patients carrying the P413L human chromogranin B (hCgB) variant develops disease nearly ten years earlier than ALS patients without this variation (Gros-Louis F, et al. 2009). The P413L hCgB is the first genetic variation documented to influence the age of onset in both fALS and sALS. Here, we further investigated the role of hCgB variants as modifier of disease onset and progression for ALS. Cell transfection experiments demonstrated the defective ability of hCgB variants to bind to mutant SOD1 and the defective sorting and maturation of hCgB variants into secretory granules. We generated transgenic mice overexpressing genomic fragments encoding the hCgB variants and crossed them to G37R-SOD1 mice to examine the effects of hCgB variants in mutant SOD1-mediated disease. So far, our analyses of Kaplan-Meier curves for disease onset and for survival of doubly Tg mice co-expressing mutant SOD1 with either P413 hCgB or L413 hCgB variants are consistent with the view that hCgB expression level and polymorphism are factors that may influence ALS disease onset or duration.

Disclosures: Y. Ohta: None. **D. Phaneuf:** None. **J. Julien:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.06/M7

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH1 R21 NS054934-01A1

Forbes Norris MDA/ALS Research Center

NSFC#81071010

Title: Investigation of the role carbonic anhydrase 1 plays in motor neuron function and ALS pathology

Authors: *J. LIU[1], X. LIU[1], R. P. BOWSER[2], R. G. MILLER[3], T. KADOWAKI[1]; [1]Deprtment of Biol. Sci., Xi'An Jiao-Tong Liverpool Univ., Jiangsu, China; [2]Barrow Neurolog. Inst., Phoenix, AZ; [3]Forbes Norris MDA/ALS Res. Ctr., San Francisco, CA

Abstract: Carbonic anhydrase I (CA1) is the cytosolic form of the 16 isoforms of α-CA family in mammalian cells. CA is a zinc metalloenzyme that catalyzes the reversible conversion of CO_2 and H_2O to HCO_3^- and H^+ and is important in maintaining cellular pH homeostasis. CA2-deficiency in human causes Marble Brain syndrome with symptoms of osteopetrosis, renal tubular acidosis, and mental retardation. Other forms of CA including the membrane-associated CA9 and mitochondrion-resident CA5 have been indicated in cancer and obesity, respectively. Whether and how CA1 mightbe involved in ALS pathology is completely unknown. The objective of the study is to determine CA1 expression in human spinal cord and further investigate the potential mechanisms by which CA1 might be involved in ALS pathology. Methods: Immuohistochemical and/or immunofluorescent staining were used with human spinal cord section. Subcellular fractionation method was used to extract cytosolic and membrane-bound CA1s from spinal cord tissues. N2a-stable cell line was established to express human CA1 and Western analysis was carried out to examine ER stress responses. Transgenic *Drosophila* line of UAS-CA1 construct was generated by BestGeneInc. GMR and D42-GAL4 lines were purchased from Bloomington *Drosophila* Stock Center at Indiana University. Results: 1) CA1 protein level is significantly decreased in ALS spinal cord; 2) For the first time, we show that CA1 is expressed in human spinal cord motor neurons while CA2 is expressed outside the motor neurons; 3) In addition, CA1-immnuohistochemical staining in the motor neurons exhibit punctated instead of the diffused pattern as predicted. Further staining with subcellular organelle markers demonstrates the colocalization of CA1 with an ER-lumen protein PDI; 4) Biochemically, membrane-associated CA1 is increased to a greater degree than the cytosolic CA1 in ALS spinal cord. In contrast, CA2 is not changed in either the cytosolic or membrane-associated fraction between ALS and control subjects; 5) In N2a cells, expression of human CA1 decreases mutant SOD1-induced ER stress marker proteins BiP and phosphorylated eIF2α; 6) In *Drosophila*, eye-specific expression of human CA1 results in no observable phenotype while motor neuron-specific expression causes 54% reduction in the number of the hatched embryos. Conclusion: our studies suggest that CA1 may have important regulatory function in the motor neurons. One potential mechanism for CA1's involvement in ALS pathology can be through the ER stress-response pathway.

Disclosures: J. Liu: None. **R.P. Bowser:** None. **R.G. Miller:** None. **T. Kadowaki:** None. **X. Liu:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.07/M8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Hermann und Lilly Schilling-Stiftung

Bundesministerium für Bildung und Forschung (BMBF)

Title: Compartmentalized motoneuron cultures reveal alterations in axonal mRNAs after TDP-43 depletion

Authors: *L. SAAL[1], M. BRIESE[1], S. KNEITZ[2], M. SENDTNER[1];
[1]Inst. for Clin. Neurobio., Wuerzburg, Germany; [2]Dept. of Physiological Chem. I, Theodor-Boveri-Institute for Biol. Sciences, Univ. of Wuerzburg, Wuerzburg, Germany

Abstract: Compartmentalized culture systems are ideal tools for studying local signaling mechanisms including differential RNA distribution in axonal and somatodendritic compartments of neurons. We developed a technique for culturing primary mouse motoneurons in microfluidic chambers to investigate changes in levels and distribution of axonal transcripts after TDP-43 depletion. Gradients of the brain derived neurotrophic factor (BDNF) were used to direct axon growth into a separate culture compartment. With this technique we studied the effects of TDP-43 suppression using lentiviral shRNA knockdown. After 7 DIV the RNA of the cellbody compartment and the axonal compartment was extracted, linearly amplified and hybridized on a 3'IVT Affymetrix Gene Chip® Mouse Array. Analysis of these microarray data shows a massive downregulation of specific axonal RNAs after TDP-43 depletion. Because the expression levels of these RNAs are unchanged in the somatodendritic compartment and only reduced in the axonal compartment, this indicates that, in addition to its well-characterized function in splicing, TDP-43 plays a role in axonal translocation of specific mRNAs, coding for proteins that are relevant for axonal and presynaptic differentiation and function. This is consistent with the observed phenotypes of shorter axons after TDP-43 knockdown in cultured motoneurons.

Disclosures: L. Saal: None. **M. Briese:** None. **S. Kneitz:** None. **M. Sendtner:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.08/M9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Mutant SOD1 astrocytes display an accelerated aging phenotype in amyotrophic lateral sclerosis

Authors: *M. DAS, C. SVENDSEN;
RMI, Cedars Sinai Med. Ctr., Los Angeles, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized primarily by the death of motor neurons in the brain and spinal cord. However, ALS is not a cell autonomous disease; the glial microenvironment can significantly modulate motor neuron survival. Astrocytes with an ALS causing mutation such as in the protein superoxide dismutase I (SOD1) are detrimental to motor neuron survival. While many have embarked on the quest for the mechanism through which astrocyte-mediated toxicity occurs, the answer remains elusive.

The prevalence of amyotrophic lateral sclerosis (ALS) increases with age. However, the possible interaction between the normal degenerative aging process and the rapid cell loss in ALS has not been thoroughly investigated. Here, we find that compared to astrocytes from young rats, astrocytes from aged wildtype rats have a reduced ability to support motor neurons in culture. To investigate an explanation for age-related differences between astrocytes we look at cell senescence. Cellular senescence is a consequence of aging in many tissue types and the accumulation of these non-proliferating cells leads to tissue atrophy. We observe that aged astrocytes contain a higher proportion of senescent cells relative to young astrocytes. Surprisingly, we find that astrocytes from an ALS rat develop a large population of cells in a senescent state at younger age than wildtype rats. ALS astrocytes display a significantly higher proportion of senescent cells compared to an age-matched wildtype counterpart. These data suggest that a mechanism long associated with aging is amplified in the presence of toxic protein stress. Identifying the cellular changes during aging that influence neurodegenerative disease can help us gain insight into identifying new targets to combat ALS.

Glial-derived neurotrophic factor (GDNF) is a protein shown to directly promote motor neuron survival. Here, we show that GDNF can alter astrocyte gene expression. We find that exogenous addition of GDNF onto astrocytes can reduce p21 expression and decrease senescence associated cytokine secretion. Hence, we have found a novel method to modulate the environment of aged and SOD1 motor neurons.

Disclosures: M. Das: None. **C. Svendsen:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.09/M10

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: MDA development grant

Title: Targeting misfolded SOD1 as a therapy for ALS

Authors: *A. ISRAELSON[1], D. W. CLEVELAND[2];
[1]Ben Gurion Univ., Beer Sheva, Israel; [2]Ludwig Inst. for Cancer Res., La Jolla, CA

Abstract:
Amyotrophic lateral sclerosis (ALS) is a late-onset fatal neurodegenerative disease characterized by the loss of upper and lower motor neurons. The reason for the degeneration of motor neurons in ALS is still unknown. Mitochondria have been implicated as a possible target for toxicity by several studies reporting a range of dysfunctions and the toxic binding of misfolded SOD1 to mitochondrial targets. However, the mechanism by which mutant SOD1 associates with mitochondria specifically from affected tissues is still unknown. Preliminary data show that a cytosolic factor in unaffected tissues is responsible for preventing the accumulation of misfolded SOD1. Using mass spectrometry, this factor was identified as a multifunctional protein with activities as an intracellular chaperone. The factor directly inhibits mutant SOD1 binding to mitochondria and misfolded SOD1 is suppressed by increased expression of that chaperone in neuronal cells. Antisense oligonucleotide-mediated reduction of the newly identified chaperone in peripheral tissues of mutant SOD1-expressing rats induces misfolded SOD1, demonstrating that the endogenous chaperone suppresses misfolded SOD1 accumulation. In view of all the evidence to date, we believe that it is possible that the neurodegeneration observed in ALS is a result of a reduction or malfunction of specific chaperones, leading to accumulation and toxicity of misfolded SOD1. We suggest that finding these pathways and identifying this chaperone for misfolded SOD1 will make a positive contribution for the future development of ALS therapies.

Disclosures: A. Israelson: None. **D.W. Cleveland:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.10/M11

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Evidence for a dying-forward process of ALS in the SOD1 rat

Authors: *G. M. THOMSEN[1], G. GOWING[1], P. AVALOS[1], J. LATTER[1], K. STAGGENBORG[1], R. PARADIS[1], M. CHEN[1], A. LIN[1], B. KASPAR[2], C. SVENDSEN[1]; [1]Cedars Sinai Med. Ctr., West Hollywood, CA; [2]Nationwide Children's Res. Institute, Ohio State Univ. Sch. of Med., Columbus, OH

Abstract: The mechanisms underlying the causality of motor neuron death in Amyotrophic Lateral Sclerosis (ALS) have yet to be elucidated. Conflicting reports exist in support of two main hypotheses: (i) The "dying-forward" hypothesis proposes that ALS is mainly a disorder of cortical motor neurons, which connect with anterior horn cells of the spinal cord mediating anterograde degeneration of these cells leading to muscle degeneration. (ii) The "dying-back" hypothesis proposes that ALS begins at the neuromuscular junction. These hypotheses are largely unexplored in the hSOD1(G93A) rat model of ALS. Here, we evaluated the degree and timing of degeneration in the cortex, spinal cord, distal (L5 ventral root) axons and muscle in pre-symptomatic, early symptomatic and endpoint SOD1 rats. We provide evidence that the degenerative process in SOD1 rats begins in upper motor neurons and not in the muscle, thereby supporting the "dying-forward" hypothesis of ALS. We found that the first sign of alterations in these rats occurred in the cortex whereby CTIP2+ corticospinal motor neurons in layer V were significantly smaller than those of wildtype controls. This occurred as early as 90 days of age, prior to the onset of symptoms. Spinal motor neuron degeneration was also first observed pre-symptomatically at p120, at which point total numbers of large ChAT+ neurons were significantly decreased. On the other hand, there were only minimal signs of distal axonal degeneration at p120 and nearly all (>95%) of neuromuscular junctions were still fully innervated at this time point. Significant denervation of muscle did not occur until the early symptomatic time point, at which point nearly 75% of NMJs had become denervated. We also injected pseudorabies virus, a retrograde tracer, into the gastrocnemius at various time points to examine the circuitry between muscle and cortex in SOD1 rats. After quantifying retrogradely labeled corticospinal motor neurons, we found that at p120, but not at p90, there were significantly fewer labeled cortical layer V neurons. Although neuromuscular junctions appear healthy at presymptomatic time points, this suggests that there is an early disconnect within the circuitry of SOD1 rats. Further analyses including examination of labeled spinal motor neurons are ongoing to determine the origin of this disconnect. Additionally, studies are underway to further test the dying-forward hypothesis in the SOD1 rat model using AAV9-siRNA to knock down SOD1 expression in the brain and spinal cord. Studies such as these will provide insight into the origin of degeneration of ALS in order to develop potential therapeutic approaches.

Disclosures: G.M. Thomsen: None. **G. Gowing:** None. **P. Avalos:** None. **J. Latter:** None. **K. Staggenborg:** None. **R. Paradis:** None. **M. Chen:** None. **A. Lin:** None. **C. Svendsen:** None. **B. Kaspar:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.11/M12

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Grant-in-Aid for Scientific Research (A) from Japan Society for the Promotion of Science

Grant-in-Aid for the Research Committee of CNS Degenerative Diseases from Ministry of Health, Labor and Welfare, Japan

Title: TARDBP mutations associated with amyotrophic lateral sclerosis alter the efficiency of its own alternative splicing

Authors: T. KONNO, A. KOYAMA, M. KOYAMA, A. SUGAI, T. KATO, *T. ISHIHARA, M. NISHIZAWA, O. ONODERA;
Niigata Univ. Resource Br. For Brain Dis., Niigata, Japan

Abstract: Background: TAR DNA-binding protein 43 (TDP-43), encoded by TARDBP, is a major component of neuronal cytoplasmic inclusions, which are observed in affected neurons in patients with amyotrophic lateral sclerosis (ALS). To date ~40 mutations in TARDBP were identified in patients with ALS, and most of them located in exon 6, the last exon of TARDBP. It is known that TDP-43 regulates its own protein level through binding TARDBP pre-mRNA and introducing intra-exonic alternative splicing in exon 6, and produces splicing isoforms with a premature termination codon. They are supposed to be degraded by nonsense-mediated mRNA decay. Given the fact that the mutations in TARDBP are concentrated in exon 6, we speculate that the mutations affect the alternative splicing.
Object: To investigate whether mutations associated with ALS alter the efficiency of its own alternative splicing.
Materials and Methods: We generated minigenes containing wild-type and mutant (G295S, G298S, A315T, M337V, Q343R, G348C and A382T) TARDBP exon 6 with flanking intron sequence. Next, to assess the effect of mutations on cis-acting RNA sequence motifs, we transiently transfected these minigenes into HEK293T cells with or without wild-type TDP-43. In addition, to assess whether mutant TDP-43 cause mis-splicing of TARDBP pre-mRNA by acting as trans-acting factor, we transiently co-transfected mutant TDP-43 and wild-type minigene into HEK293T cells. We extracted RNA from cells at 48 hours after transfection. We conducted reverse-transcription polymerase chain reaction using minigene specific primers and

gel electrophoresis to evaluate the alternative splicing.
Results: Wild-type TDP-43 efficiently produced intra-exonic splicing isoforms from wild-type minigene. We found that wild-type TDP-43 less efficiently produced the splicing isoforms from some mutant minigenes than wild-type minigene. In contrast, mutant TDP-43 protein produced the splicing isoforms comparable to those of wild-type TDP-43.
Discussion: These results indicated that the mutations in TARDBP could alter the efficiency of the alternative splicing. The investigation of these findings in neuronal cells or the affected tissue from patients with ALS would be interesting.

Disclosures: T. Konno: None. **T. Ishihara:** None. **A. Koyama:** None. **M. Koyama:** None. **A. Sugai:** None. **T. Kato:** None. **M. Nishizawa:** None. **O. Onodera:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.12/M13

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Grant-in-Aid for Scientific Research (A) from Japan Society for the Promotion of Science

Grant-in-Aid for the Research Committee of CNS Degenerative Diseases from Ministry of Health, Labor and Welfare, Japan

Title: Alternative splicing or polyadenylation, which is the major mechanism for auto-regulation of TDP-43?

Authors: A. SUGAI, A. KOYAMA, T. KATO, T. KONNO, T. ISHIHARA, M. NISHIZAWA, *O. ONODERA;
Brain Res. Institute, Niig, Niigata, Japan

Abstract: Background: TAR DNA binding protein-43 (TDP-43), predominantly nuclear protein, plays a key role in pathogenesis of amyotrophic lateral sclerosis. Accumulating evidence suggest that TDP-43 levels in nucleus should be strictly regulated especially in central nervous system. TDP-43 is auto-regulated via binding to its own 3'UTR. Following mechanism of the auto-regulation, however, remains unclear.
Objective: To investigate the mechanism of TDP-43 auto-regulation.
Materials and Methods: Flp-In 293 cell lines stably expressing myc-tagged wild-type TDP-43 cDNA was used to investigate elevated TDP-43 effects on endogenous TDP-43 mRNA.

Minigene containing wild-type exon-6 of TDP-43, which includes TDP-43 binding region, was used to investigate depleted TDP-43 effects on the transcripts. In situ hybridization, northern blotting of polyA (+) RNA in cytoplasmic and nuclear extraction, and 3'-end qRT-PCR were performed to evaluate distribution and expression levels of each isoform of the transcripts.
Results: Northern blot analysis and qRT-PCR revealed that TDP-43 mRNA was alternatively polyadenylated. The levels of transcripts using distal polyadenylation sites were increased with increasing TDP-43 levels. In situ hybridization and northern blot analysis of RNA extracted from nucleus or cytoplasm showed that substantial amounts of TDP-43 mRNA with distal polyadenylation were located in nucleus. The increasing level of TDP-43 reduced its own mRNA from cytoplasm, whereas the TDP-43 mRNAs in nucleus with distal polyadenylation sites were unchanged. The mRNA with different polyadenylation sites, however, did not alter its stability. Northern blot analysis upon cycloheximide treatment showed shorter isoforms, excised two or three introns in exon-6. These isoforms fulfilled the criteria for nonsense-mediated mRNA decay (NMD).
Conclusion: Increasing TDP-43 undergo excision of intra-exonic introns, resulting in reduction of its own mRNA via NMD pathway. In addition, the amounts of TDP-43 altered the polyadenylation sites in combination with alternative splicing, which regulates intracellular distribution (nuclear or cytoplasm) of the mRNA. These mechanisms are collaboratively involved in the auto-regulation of TDP-43.

Disclosures: A. Sugai: None. **O. Onodera:** None. **A. Koyama:** None. **T. Kato:** None. **T. Konno:** None. **T. Ishihara:** None. **M. Nishizawa:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.13/M14

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH-NINDS R01NS078375

R01NS069601

W81XWH-11-1-0689

SMA Foundation

FSMA A. Lewis Young Investigator Award

Title: A stem cell model of the motor circuit reveals distinct requirements of SMN for motor neuron survival and function

Authors: *C. M. SIMON, A. JANAS, F. LOTTI, L. PELLIZZONI, G. MENTIS;
Col. of Physicians and Surgeons, Motor Neuron Ctr., New York, NY

Abstract: Spinal muscular atrophy (SMA) is a motor neuron disease caused by deficiency in the ubiquitously expressed survival motor neuron (SMN) protein. Although a hallmark of the disease is the death of motor neurons, studies from animal models revealed that neuronal circuit perturbations are important determinants of SMA pathogenesis that precede motor neuron loss. It is currently unknown if motor neuron death and circuit dysfunction are linked events induced by SMN deficiency. To address this, we have generated mouse ES (Hb9::GFP) cell lines with regulated RNAi knockdown of endogenous SMN and established a simplified *in vitro* model of motor circuits based on the use of ES-derived motor neurons (MNs) and interneurons (INTs). MNs differentiated from ES (Hb9::GFP) cells express GFP under the control of the MN-specific Hb9 promoter and their survival over time was measured using whole-well automated imaging in 96 well format. This time-course analysis revealed significantly decreased survival of SMN-deficient MNs relative to controls. To investigate whether MN death induced by SMN deficiency was a cell autonomous process, we purified ES-MNs by FACS. We found that the rate of death of purified SMN-deficient ES-MNs does not significantly differ from that of SMN-deficient MNs co-cultured with INTs. In addition, SMN deficiency leads to preferential loss of MNs compared to INTs.
ES-derived INTs are both excitatory (VGluT2,3) and inhibitory (VGAT, GlyT2) and form synapses onto the soma and dendrites of MNs. To study SMN requirement for motor neuron function in our *in vitro* motor circuit, we employed intracellular patch clamp recordings. SMN-deficient MNs exhibited an increase in the passive membrane properties compared to MNs with normal SMN levels. To determine if the hyperexcitability of SMN-deficient MNs was cell-autonomous, we recorded from FACS purified ES-MNs and found that SMN deficiency had no effects on the intrinsic properties of MNs cultured in isolation. To quantify MN output, we measured the spontaneous firing frequency. SMN deficiency caused a significant reduction in the firing frequency of MNs co-cultured with INTs compared to normal controls.
These results show that neuronal death induced by SMN deficiency occurs via cell-autonomous mechanisms and preferentially affects MNs. In contrast, they reveal a non-cell autonomous origin for the SMN-dependent changes in the spontaneous activity and intrinsic excitability of MNs. Collectively, our study suggests that dysfunction and death of motor neurons are distinct events that occur downstream of SMN deficiency as a result of differential effects in specific neuronal types.

Disclosures: C.M. Simon: None. **A. Janas:** None. **F. Lotti:** None. **L. Pellizzoni:** None. **G. Mentis:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.14/M15

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01-NS074886

NIH Grant F31NS080539

Title: Astrocytes harboring Amyotrophic Lateral Sclerosis-causative mutations alter ABC drug efflux transporters at the endothelial cell layer

Authors: *M. R. JABLONSKI, D. A. JACOB, P. PASINELLI, D. TROTTI;
Thomas Jefferson Univ., PHILADELPHIA, PA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a slowly progressing neurodegenerative disease, characterized by motor neuron degeneration. ALS is a non-cell-autonomous disease with astrocytes contributing to toxicity and selective loss of motor neurons. The blood-brain barrier (BBB) is formed by endothelial cells in association with pericytes and astrocytes, forming the neurovascular unit. Astrocytic end-feet encapsulate approximately 90% of endothelial cells and maintain homeostasis of the barrier. ABC drug efflux transporters, highly localized in endothelial cells, prevent a wide range of neurotoxicants and therapeutics from entering the CNS. We recently reported a disease-driven increase in expression and function of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in ALS mouse spinal cord capillaries and expression increases in human spinal cord tissue (Jablonski et al. 2012; Neurobiol Dis), suggesting these transporters could mediate ALS pharmacoresistance. Due to the toxic contribution by astrocytes in ALS, we hypothesized that toxic astrocytes impart increases in ABC transporters in endothelial cells, leading to a decrease in bioavailability of therapeutics in the CNS. To examine this hypothesis, we implemented a co-culture system whereby primary cultured mouse brain endothelial cells or the mouse brain endothelioma cell line, bEnd.3, were plated on a transwell above a layer of primary mouse astrocytes. Astrocytes were either oxidatively stressed, indicative of a potential mechanism of neurodegeneration in ALS, or infected with virus expressing wild-type or an ALS-causative mutant (G93A) superoxide dismutase-1 (SOD-1). Oxidatively stressed astrocytes increased P-gp expression dose-dependently in bEnd.3 cells. SOD1-G93A virus-treated astrocytes significantly increased P-gp expression levels in bEnd.3 cells and primary cultured endothelial cells compared to astrocytes treated with wild-type SOD1 virus. NFkB inhibition (SN50) in endothelial cells and bEnd.3 cells prevented the endothelial P-gp expression increases seen with oxidatively stressed or SOD1-G93A-containing astrocytes. This project is an important step in understanding drug efflux transporter regulation in ALS.

Disclosures: M.R. Jablonski: None. **D.A. Jacob:** None. **P. Pasinelli:** None. **D. Trotti:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.15/M16

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH-NINDS Grant RO1-NS065895

NIH-NINDS Grant 1F31NS076250-01A1

Title: SMA skeletal muscles in primary cell culture have normal morphology, survival, growth, and response to DNA damage

Authors: *S. FAYZULLINA, L. J. MARTIN;
Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: We previously showed that spinal muscular atrophy (SMA) mice exhibit skeletal muscle DNA damage and cell death before major spinal cord motor neuron cell body and axon degeneration. We investigated whether the observed DNA damage is due to faulty DNA repair in SMA skeletal muscle. Using a mouse model of SMA (Hsieh-Li et al. 2000), we established primary myoblast cultures from the skeletal muscles of SMA mice and control littermates. Myoblast cultures were then differentiated into myotubes. Myotube differentiation and growth was assessed by measuring myotube length and counting myonuclei. DNA damage was induced by gamma-irradiation, etoposide, and methane methylsulfonate (MMS). DNA damage was detected by the comet assay. DNA damage response was assessed by phospho-p53 (S15) and γH2AX immunofluorescence.
SMA mice had smaller muscle mass than littermate control mice at harvest, but cultures seeded at equal cell density resulted in myotube density similar to control myotubes. Myotubes cultured from SMA mice at P5 had similar morphology, multinucleation, and length, compared with control myotubes. Gamma-irradiation (5 Gy) induced comparable levels of DNA damage in SMA and control myotubes, as indicated by the distribution of comet scores. After a 1 hour recovery period, both SMA and control myotubes repaired most of the induced damage. Etoposide treatment (10 μM for 1 hour) yielded similar results. MMS (100 μg/mL) induced similar DNA damage in SMA and control myotubes. SMA myotubes were not more sensitive to a low dose of MMS (1 μg/mL) than controls. In both SMA and control myotubes, without induction of DNA damage, we observed discrete, colocalized foci of γH2AX and phospho-p53 in myotube nuclei. DNA damage, induced by either irradiation or etoposide, caused dispersal of

foci within the nucleus. After a 1 hour recovery period, the nuclear foci re-assembled in most myotube nuclei. Both dispersion and re-assembly of nuclear foci upon recovery were similar in SMA and control myotubes.
We observed that primary skeletal muscle cells cultured from SMA mice exhibit myotube formation and growth comparable to control cultures. SMA and control myotubes have similar sensitivity to DNA damage, induced by either irradiation or chemical agents. SMA myotubes exhibit a normal response to DNA damage and functional DNA repair. These observations indicate that the DNA damage and cell death observed in skeletal muscle of SMA mice is unlikely to be due to faulty autonomous DNA repair mechanisms or differentiation. Our observations indicate that the marked prenatal DNA damage accumulation in skeletal muscle of SMA mice is likely a consequence, rather than a primary cause, of myocyte cell death.

Disclosures: S. Fayzullina: None. **L.J. Martin:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.16/M17

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH-NINDS

R01NS078375

DOD

W81XWH-11-1-0689

SMA Foundation

FSMA, A. Lewis Young Investigator Award (G.Z.M.)

Title: Non-cell autonomous mechanisms induce hyperexcitability of motor neurons in a mouse model of spinal muscular atrophy

Authors: *E. FLETCHER, G. Z. MENTIS;
The Ctr. for Motor Neuron Biol. and Dis., Columbia Univ., New York, NY

Abstract: Spinal muscular atrophy (SMA) is a severe neurodegenerative disease caused by reduced levels of the survival motor neuron (SMN) protein. The hallmarks of SMA are loss of

motor neurons, muscle atrophy and abnormal postural reflexes. However, the mechanisms by which a reduction in SMN protein leads to selective motor deficits and muscle weakness are poorly understood. Using the SMA-Δ7 mouse model, we have previously shown that sensory-motor circuit dysfunction occurs early in development and precedes motor neuron loss in the course of the disease. At post-natal day 4 (P4), L1-L2 lumbar motor neurons innervating proximal muscles are abnormally hyperexcitable and exhibit reduced stretch reflexes compared with L5 motor neurons innervating distal hindlimb muscles which are affected at a later stage. To address whether this hyperexcitability is due to premotor synaptic dysfunction or due to SMN deficiency *per se* in motor neurons, we investigated the intrinsic properties of both: i) L1-L3 motor neurons at P2 and ii) L5 motor neurons at P4.

We employed the intact *in vitro* spinal cord preparation from age-matched wild type (wt) and SMA-Δ7 mutant mice. We measured the passive membrane properties of input resistance (R_{IN}) and time constant (τ) as well as the rheobase current using whole-cell patch clamp recordings from motor neurons, identified by ventral root antidromic stimulation. Our preliminary results reveal that L1-L3 SMA motor neurons at P2 were significantly hyperexcitable compared with their wt counterparts for all three parameters tested (R_{IN}: 67.9±12.5MΩ in wt, n=8, and 171.0±28.7 MΩ in SMA, n=6; $P<0.01$, t-test. τ: 10.1±1.2ms in wt and 17.8±2.5ms in SMA, $P<0.001$, t-test). At the same time, the monosynaptically-evoked EPSPs following proprioceptive fibers stimulation revealed a significant reduction. The intrinsic properties of L5 motor neurons at P4 however, did not reveal any significant alterations for the same intrinsic membrane properties. As expected, the monosynaptic EPSPs were similar between wt and SMA mutants in L5 motor neurons at P4.

These findings indicate that SMN deficiency alters the intrinsic excitability of SMA motor neurons through non-cell autonomous mechanisms resulting from spinal circuit dysfunction, consistent with SMA being a disease of motor circuits.

Disclosures: E. Fletcher: None. **G.Z. Mentis:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.17/M18

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Packard Center Grant

NIH RO1 Grant NS 047484

Title: Dysregulation of atypical PKC and Ryk in a mouse model of amyotrophic lateral sclerosis

Authors: *A. TURY, K. TOLENTINO, A. FENSTERMAKER, R. MCRAE, Y. ZOU;
Div. of Biol. Sciences, Section of Neurobio., UCSD, La Jolla, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastative neurodegenerative disease characterized by the degeneration of lower and upper motor neurons, with no available cure. The primary causes of motor neuron degeneration are still unknown. Increasing evidence indicates that Wnt signaling is dysregulated in neurodegenerative diseases, including ALS. In this study, we focused on two non-canonical Wnt signaling components, atypical PKC (aPKC) and Ryk. aPKC mediates Wnt signaling to regulate growth cone guidance, axon differentiation and cell death/survival. Ryk is a repulsive receptor for Wnts that is re-expressed and inhibits regeneration after spinal cord injury and our preliminary results suggest that Ryk reduces neuronal cell death triggered by loss of Frizzled 3. We found that aPKC expression was increased in motor neurons of the ventral lumbar spinal cord in a mouse model of ALS (SOD1-G93A) at early and late stages of the disease compared to SOD1-WT mice. aPKC upregulation may either contribute to the disease or may be a failing attempt to protect motor neurons from degeneration. We found that aPKC activity measured by the level of autophosphorylation of aPKC by western blotting was reduced. The ratio of total aPKC was increased in the detergent insoluble fraction in SOD1-G93A mice and aPKC colocalized with SOD1 aggregates. In addition, we found that aPKC aggregates increased in the ventral horn of the spinal cord with disease progression. These results suggest that aPKC may be trapped in mutant SOD1 aggregates and not be able to function properly to protect motor neurons from death. We also found that Ryk expression was increased in the motor neurons and the ventral white matter in the ventral lumbar spinal cord of mutant SOD1 mice at different stages, with a peak of increase in motor neurons at early stage. One hypothesis is that Ryk might be involved in the early events that triggers axonal and motor neuron degeneration in the spinal cord of ALS mice. These studies suggest that Wnt/aPKC and Wnt/Ryk signaling may be potential targets for treatment of ALS.

Disclosures: A. Tury: None. **K. Tolentino:** None. **A. Fenstermaker:** None. **R. McRae:** None. **Y. Zou:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.18/N1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: JSPS KAKENHI Grant Number 23890060

Title: Reduction of U11/U12 small nuclear ribonucleoprotein in amyotrophic lateral sclerosis

Authors: T. ISHIHARA, T. KATO, A. SHIGA, *A. YOKOSEKI, A. KAKITA, M. NISHIZAWA, H. TAKAHASHI, O. ONODERA;
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Abstract: OBJECTIVE:To investigate 1) whether the repertoires of U11/U12 spliceosome alter in affected neuronal tissues with amyotrophic lateral sclerosis (ALS), 2) whether pre-mRNA splicing associates with U11/U12 spliceosome alters in ALS affected tissues.
INTRODUCTION: TAR-DNA Binding Protein 43kDa (TDP-43) has crucial role in the pathogenesis of ALS. TDP-43 is one of the heterogeneous nuclear ribonucleoproteins and co-localizes with GEM, which is one of the nuclear bodies composed chiefly of survival of motor neurons (SMN). SMN is a causative protein for spinal muscular atrophy, an infantile onset motor neuron disease. SMN plays an important role in maturation of snRNAs which are components of spliceosome. Spliceosome is the machinery that carries out pre-mRNA splicing, and is classified into two types; major (U1, U2, U4, U5 and U6) and the minor (U11, U12, U5, U4atac and U6atac) spliceosomes. Depletion of SMN alters the repertoires of snRNAs by cell and tissue-type specific manner, particularly reduction of U11/U12 snRNAs in spinal cord (Zhang et al. cell.2008). We have found that U12 snRNA decreased in affected tissues with ALS (Ishihara, reported in SFN 2012). However it is unclear whether the reduction of the snRNAs alters the amounts and function of minor spliceosome.
METHODS: Spliceosome is composed of snRNAs and associated proteins complex, small nuclear ribonucletide protein (snRNP). Thus we investigated the amount of U11/12 type spliceosome in spinal motor neurons with ALS patients and controls (n=4) by immunofluorescent staining with antibody to snRNP 59 kDa, which is a component of minor spliceosome. Quantitative real-time RT-PCR of snRNAs and splicing efficiency of pre-mRNA were performed with RNA from cultured cells and neuronal tissues (spinal cord, motor cortex and cerebellum as tissues with or without TDP-43 pathology) from ALS patients (n=7-10) and control individuals (n=9-10). Total RNA was extracted using mirVana miRNA isolation kit (Ambion).
RESULTS: The fluorescence intensity of snRNP 59K was decreased to 39% in spinal motor neuron with ALS compared with control. The level of mRNA in which minor spliceosome dependent intron was included significantly increased in TDP-43 depleted U-87 MG cells (218%), and the same results were obtained in the motor cortex with ALS but not in spinal cord and cerebellum.
DISCUSSION: The decreased amounts of snRNP 59K indicate that the reduction of U12 snRNA reduces the level of minor spliceosome. In addition, some minor spliceosome dependent introns were retained in affected tissue with ALS. Our results suggest that decreasing the function of minor spliceosome may underline the molecular pathogenesis of ALS.

Disclosures: T. Ishihara: None. **T. Kato:** None. **A. Shiga:** None. **A. Yokoseki:** None. **A. Kakita:** None. **M. Nishizawa:** None. **H. Takahashi:** None. **O. Onodera:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.19/N2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Paul and Harriett Campbell Fund for ALS Research

Zimmerman Family Love Fund

ALS Association, Greater Philadelphia Chapter

Title: Plasma biomarker panels predict prognosis in amyotrophic lateral sclerosis

Authors: *X. W. SU[1], Z. SIMMONS[2], R. M. MITCHELL[1], H. E. STEPHENS[2], J. R. CONNOR[1];
[1]Neurosurg., [2]Neurol., Penn State Col. of Med., Hershey, PA

Abstract: Background: It is difficult to predict prognosis in ALS. Although median survival is 2-4 years, survival ranges from months to decades. This uncertainty complicates clinical management and outcomes assessment associated with treatment trials.
Objectives: To identify panels of plasma cytokines, trophic factors and metabolites with prognostic value in ALS, and to use novel multivariate modeling strategies incorporating these panels to predict disease prognosis.
Methods: An IRB-approved, retrospective analysis of plasma from 26 sporadic ALS patients seen at a university-based multidisciplinary ALS clinic was conducted. Plasma was analyzed with multiplex and ELISA assays as well as clinical laboratory procedures. Multiplex and ELISA assays measured levels of 27 and 8 biomarkers, respectively. Gender, H63D HFE status and site of onset were included as categorical variables. Clinical measures, including disease duration, rates of decline in forced vital capacity (FVC) and ALS Functional Rating Scale-Revised (ALSFRS-R) scores, and time to non-invasive ventilation (NIV), served as dependent variables. Statistical modeling used hierarchal forward, main effects only multivariate regression with stepwise switching. Goodness-of-fit validation and power analyses were performed. Individual subject-level 95% confidence bands for predictions were constructed.
Results: Models for disease duration, rates of decline in FVC and ALSFRS-R scores, and time to NIV incorporated six, four, three, and five predictive biomarkers to achieve R-squared values of

0.820, 0.723, 0.640 and 0.913, respectively. Inflammatory cytokines, including select interleukins (IL), and indicators of iron metabolism, including transferrin and transferrin saturation, had predictive value. IL-1β, eotaxin and transferrin were significant predictors in multiple models. After classification of disease duration into categories, all predictions were within one level of actual outcome, with 10 out of 11 patients with slow progression (survival more than four years) predicted to have less aggressive disease. Select biomarkers were consistent with previous reports, including IL-1β and RANTES as negative predictors, and granulocyte-colony stimulating factor as a positive predictor. Bulbar onset was associated with higher rates of decline in FVC, as expected given the difficulty for these patients to complete FVC measurements, highlighting the validity of results.
Conclusions: These results support plasma biomarker-based predictive modeling of ALS prognosis, and provide a rationale for unbiased biomarker discovery and longitudinal follow-up in larger patient populations.

Disclosures: X.W. Su: None. **Z. Simmons:** None. **R.M. Mitchell:** None. **H.E. Stephens:** None. **J.R. Connor:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.20/N3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Thr175 phosphorylation regulates GSK3β activity and tau fibril formation *In vitro*

Authors: A. MOSZCZYNSKI[1], M. GOHAR[1], K. VOLKENING[1], *M. J. STRONG[2]; [1]Mol. Med., Robarts Res. Inst., London, ON, Canada; [2]London Hlth. Sci. Ctr. - UH, London, ON, Canada

Abstract: Background: Amyotrophic lateral sclerosis (ALS) is an untreatable, progressive neurodegenerative disorder defined by upper and lower motor neuron loss. Up to half of all ALS patients develop cognitive impairment (ALSci) which we have shown to be characterized by pathological fibril formation of microtubule associated protein tau. Phosphorylation of Thr175 (pThr175) has been observed specifically in ALSci, and can induce fibril formation and increased cell death in vitro. Threonine175 phosphorylation has been linked to increased GSK3β activation in vivo. In this study, we have examined whether the presence of pThr175 leads directly to the activation of GSK3β as a determinant of pathological fibril formation.
Methods: HEK293T cells were transiently transfected with either wild-type (wt) or

pseudophosphorylated (Thr175Asp) GFP-tagged 2N4R tau, or with GFP-tagged 2N4R tau in which phosphorylation at Thr175 is inhibited (Thr175Ala). Activation of GSK3β was examined by western blotting for phospho-GSK3β(Tyr216) and the effect of a panel of GSK3β inhibitors characterized with respect to their ability to inhibit GSK3β activation and cytotoxicity.
Results: Four GSK3β inhibitors were examined for toxicity at their IC50 values: Lithium chloride (LiCl; IC50 5mM) showed a 35% reduction in cell survival while AR-A014418 (100nM), Tideglusib (60nM) and TWS119 (30nM) did not show reductions in survival at their respective IC50. Both the empty vector and Thr175Ala transfected cells demonstrated similar levels of endogenous GSK3β activation, while wt-tau demonstrated a significant up-regulation of GSK3β activation. Thr175Asp transfected cells demonstrated a significant up-regulation of GSK3β activity. LiCl administration reduced GSK3β activation by 50% in wild type tau expressing cells.
Conclusions: These preliminary studies demonstrate the ability of tau to induce the activation of GSK3β in HEK293T cells. This activation appears to be dependent in part on the presence of pThr175 and can be inhibited with specific inhibitors of GSK3β . We hypothesize that Thr175 phosphorylation activates GSK3β, leading to further downstream processes culminating in tau pathology. This suggests a potential avenue of therapeutic intervention for the cognitive dysfunction of ALS.

Disclosures: A. Moszczynski: None. **M. Gohar:** None. **M.J. Strong:** None. **K. Volkening:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.21/N4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NINDS

Title: Plastin acts a genetic modifier of neurodegenerative diseases

Authors: *M. WALSH, E. WINGROVE, A. HART;
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Abstract: Spinal Muscular Atrophy (SMA) is a neurodegenerative disease caused by low levels of the Survival Motor Neuron (SMN) protein. Plastin 3 (PLS3) was identified as a human genetic modifier for SMA (Oprea *et al.*, 2008). This study reported that over-expression of PLS3 ameliorates symptoms in less severe SMA cases and PLS3 protein is in a complex with SMN in

neuronal tissues. PLS3 is an actin-bundling protein and the mechanism of SMA suppression remains unclear. Our objective is to understand the interaction between PLS3 and SMN in SMA, and to determine if PLS3 is a cross-disease modifier. Using the model system *Caenorhabditis elegans*, we examine PLS3 as a cross-species modifier of neurodegenerative disease and probe pathways involved in PLS3 suppression of SMA.
Animals with decreased levels of *C. elegans Ce*SMN-1 have neuromuscular defects in locomotion and pharyngeal pumping assays. We find that 2-fold over-expression of PLS3 (PLS3 OE) rescues neuromuscular defects in the SMA model. Ubiquitous PLS3 OE using either human or *C. elegans* plastin ameliorates the neuromuscular defects in *Cesmn-1* deficient animals. Neuron PLS3 levels are most critical as neuronal, but not muscle, expression of PLS3 rescues at high copy number. We also determined if PLS3 OE could rescue the neuromuscular defects in established *C. elegans* ALS and polyglutamine disease models. We find that PLS3 OE suppresses many of the behavioral defects in these models, demonstrating that PLS3 is a cross-disease modifier in *C. elegans*. To understand the mechanistic interaction between PLS3 and SMN, we are testing candidate genes from a list of proteins that pull-down with both SMN and PLS3 (Guruharsha et al., 2011). Thus far, we have identified several proteins/genes that act as genetic modifiers of both PLS3 OE and *Ce*SMN1 deficient animals. Further molecular and biochemical assessment is on going to determine which gene/proteins play critical roles in PLS3 suppression of SMN loss of function defects, which causes SMA.

Disclosures: M. Walsh: None. **E. Wingrove:** None. **A. Hart:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.22/N5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Ragnar Söderberg Foundation, M245/11

Birgit Backmark's Donation for ALS Research

Thierry Latran Foundation

Swedish Medical Medical Research Council 2011-2651

Åhlen's Foundation

Karolinska Institutet

Title: Protein expression with implications for selective vulnerability in motor neuron disorders

Authors: *L. H. COMLEY, I. ALLODI, S. NICHTERWITZ, A. BERGSTRAND, E. HEDLUND;
Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder, characterised by selective degeneration of the somatic motor neurons, leading to muscle wasting. Distinct populations of motor neurons show differential vulnerability to degeneration in ALS; whilst those in the oculomotor nucleus, controlling eye-movements, are generally spared, motor neurons in the hypoglossal nucleus and spinal cord are vulnerable to degeneration. To understand the reasons underlying this differential vulnerability we have analysed the global gene expression profiles of vulnerable and spared motor neurons, and identified multiple genes that show a restricted expression in vulnerable motor neurons of the hypoglossal nucleus and spinal cord. We are now analysing the expression levels of candidate genes from this screen in human brain and spinal cord tissue from non-demented controls and ALS patients. The genes being evaluated include; peripherin, a neurofilament protein, overexpression of which causes ALS-like motor neuron degeneration; dynein, a retrograde axonal transporter, mutations in which have also been shown to cause ALS-like pathology; and GABA receptor alpha2, which has a role in modulating motor neuron excitability. We anticipate that knock-down of vulnerability transcripts could confer protection to susceptible motor neurons, and provide new targets for treatments for ALS.

Disclosures: L.H. Comley: None. **I. Allodi:** None. **S. Nichterwitz:** None. **A. Bergstrand:** None. **E. Hedlund:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.23/N6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Ragnar Söderberg Foundation, M245/11

Birgit Backmark's Donation for ALS Research

Thierry Latran Foundation

Swedish Medical Medical Research Council 2011-2651

Åhlen's Foundation

Karolinska Institutet

Title: A protein signature of human motor neuron resistance in Amyotrophic Lateral Sclerosis

Authors: *S. NICHTERWITZ, L. H. COMLEY, I. ALLODI, A. BERGSTRAND, E. HEDLUND;
Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterised by the selective and progressive degeneration of somatic motor neurons. However, not all somatic motor neurons degenerate in ALS; certain groups of motor neurons, including those in the oculomotor (OM) nucleus (controlling eye movements) are spared. The reasons for this differential vulnerability among motor neurons remain largely unknown, and multiple factors are expected to be involved. Identification of mechanisms of differential motor neuron vulnerability may lead to therapies preventing the progressive loss of motor neurons in ALS.
Using a global gene expression analysis we have previously identified multiple genes that show a restricted expression in either resistant or vulnerable motor neurons in the rodent. Interestingly, several of the identified OM-restricted proteins, including parvalbumin, insulin-like growth factor II (IGF-II) and guanine deaminase (GDA), can protect vulnerable motor neurons from ALS like toxicity. We are now analysing candidate genes in human brain and spinal cord tissue from non-demented controls and ALS patients to evaluate their possible importance for human motor neuron susceptibility. Multiple genes with potential OM-restricted expression, including early growth response 1 (Egr1), guanylate cyclase 1a3 (Gucy1a3), IGF-II, GDA and Gabra1 are being evaluated. We anticipate that particular transcripts, with a preferential expression in resistant oculomotor motor neurons could be modulated to induce resistance to degeneration.

Disclosures: S. Nichterwitz: None. **L.H. Comley:** None. **I. Allodi:** None. **A. Bergstrand:** None. **E. Hedlund:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.24/N7

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant RR016475

NIH Grant GM103418

NIH Grand HD02528

NIH Grant NS078214

NIH Grant AG031575

Title: Neuromuscular alterations following unilateral isometric strength training in SOD1-G93A rats

Authors: K. G. STANFORD[1], J. D. ODUM[1], A. D. RORIE[1], R. S. ROGERS[1], J. L. WHEATLEY[1], P. C. GEIGER[1], H. NISHIMUNE[2], *J. A. STANFORD[1];
[1]Dept Molec & Integrat Physiol, [2]Anat. & Cell Biol., Univ. Kansas Med. Ctr., KANSAS CITY, KS

Abstract: Muscle use and exercise have long been implicated in the etiology and site of onset of Amyotrophic Lateral Sclerosis (ALS). This hypothesis is controversial because it is very difficult to test in humans. Preclinically, confounds inherent to conventional rodent-basedexercise protocols have also complicated interpretation. Recent studies indicate that increased AMP Kinase (AMPK) activity may worsen motor deficits in ALS. This supports the muscle use hypothesis because AMPK is activated (phosphorylated; pAMPK) during exercise. We trained SOD1-G93A rats to perform a unilateral isometric strength training task to determine if muscle strength training increases pAMPK levels and affects denervation in the trained forelimb muscles. After testing rats daily for approximately 2 months (until ~5.5 months of age), trained and untrained forelimb muscles were harvested for AMPK and pAMPK protein analysis, and for neuromuscular junction (NMJ) innervation. Our results revealed that even at endstage, SOD1-G93A rats maintained force output, although forelimb tremor was increased. Strength training increased pAMPK and protected against NMJ denvervation in trained muscles. Effects of training on proteins related to other functions (glucose transport, autophagy) were also analyzed. These results are the first to demonstrate robust neuroprotective effects of non-aerobic resistance training in ALS. They also suggest that AMPK activation may have a beneficial role in ameliorating denervation in ALS, and cast doubt on previous studies indicating a detrimental effect of AMPK activation in ALS.

Disclosures: K.G. Stanford: None. **P.C. Geiger:** None. **H. Nishimune:** None. **J.A. Stanford:** None. **R.S. Rogers:** None. **J.D. Odum:** None. **A.D. Rorie:** None. **J.L. Wheatley:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.25/N8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CI is supported by a post-doctoral grant from Fondation pour la Recherche Médicale

MMEM is supported by IHU des Neurosciences Pitié-Salpêtrière

SS is supported by a doctoral grant from Ministère de l'Enseignement Supérieur et de la Recherche

Institut pour la Recherche sur la Moelle Epinière et l'Encéphale (IRME)

Agence Nationale de la Recherche (ANR-12-JSV4-0007-01, ANR-10-IAIHU-06)

Title: Sensory abnormalities in amyotrophic lateral sclerosis: Anatomical and functional evidence in humans

Authors: *C. IGLESIAS[1], M.-M. EL MENDILI[1,2,3], S. SANGARI[4], R. MORIZOT-KOUTLIDIS[5], H. BENALI[1,2], P.-F. PRADAT[1,5], V. MARCHAND-PAUVERT[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative disease characterized by motoneurons (MNs) loss. However, spinal sensory afferents could be vulnerable as well [1,2]. Anatomical changes of the sensory ascending pathways, described using spinal magnetic resonance imaging (MRI) in recently-diagnosed ALS patients without sensory signs [3], further support this hypothesis. Yet, functional evidence is still lacking. In the present study we tested if the anatomical changes previously reported were accompanied by an altered excitability of the sensory feedback, at spinal and cortical level.
We combined spinal MRI and electrophysiological studies, in 18 ALS patients and 14 healthy subjects. Spinal MRI was done as in [3]. MRI metrics were measured at cervical levels in the lateral (motor tracts) and dorsal regions (sensory tracts). For the electrophysiology, median and ulnar nerves were stimulated electrically to activate group Ia fibers from hand-muscle (clinically altered). The subsequent somatosensory evoked potentials (SEPs) were recorded. Motor evoked potentials (MEP) were elicited in triceps brachii (clinically healthy) by transcranial magnetic stimulation (TMS) over the primary motor cortex. MEP recruitment curves were measured and MEP was conditioned by the peripheral nerve stimulations to assess the group Ia monosynaptic facilitation onto triceps' spinal MNs.
In patients, fractional anisotropy (FA) decreased in the dorsal region. In the lateral regions, FA tended to decrease while radial and mean diffusivity increased. Magnetization transfer ratio was unchanged whatever the region. The cortical SEPs' latency was unchanged but their amplitude

tended to decrease in patients. The peripheral component's amplitude (at Erb point) was reduced for both nerves. MEP recruitment curves were right-shifted in patients, with no other change, yet the maximal MEP tended to increase. This suggests an infra-clinical alteration of the cortico-motoneuronal pathway controlling the triceps. Both median- and ulnar-induced MEP facilitation tended to increase in patients. Only ulnar-effect was significant.
Though preliminary, our results suggest that early anatomical and physiological changes of the sensory feedback could occur in ALS patients. Further investigation is needed to confirm both MRI and electrophysiological results. Increased excitation onto the spinal MNs would contribute to their hyperexcitability, disturbing their homeostasis, and thus participate to the neurodegenerative process.
References
1. Kawamura et al. 1981. JNEN, 40:667-75
2. Guo et al. 2009. Exp Mol Med, 41:140-50
3. Cohen-Adad et al. 2012. ALS, 14:30-8

Disclosures: C. Iglesias: None. **M. El Mendili:** None. **S. Sangari:** None. **R. Morizot-Koutlidis:** None. **H. Benali:** None. **P. Pradat:** None. **V. Marchand-Pauvert:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.26/N9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Calcitonin gene-related peptide signaling influences motor symptom onset and disease progression in the superoxide dismutase 1 (G93A) mouse model of amyotrophic lateral sclerosis

Authors: *C. RINGER[1], K. TSUJIKAWA[2], E. WEIHE[1], B. SCHÜTZ[1];
[1]Mol. Neurosci., Philipps-Universität Marburg, Marburg, Germany; [2]Lab. of Mol. and Cell. Physiol., Osaka Univ., Osaka, Japan

Abstract: Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease affecting predominantly motor neurons in the brainstem and spinal cord, resulting in progressive paralysis and finally death. Although the exact mechanisms causing motor neuron degeneration are not fully understood, neuroinflammation contributes to disease initiation and progression. Recently, we have shown that alterations in the sub-cellular distribution of the β isoform of calcitonin gene-related peptide (CGRP) in motor neurons precede astrogliosis (Ringer et. al., 2009) and that CGRP expression levels predict motor neuron vulnerability (Ringer et al., 2012) in the

superoxide dismutase 1 (SOD1-G93A) mouse model of ALS.
To further elucidate the possible pathogenic role of CGRP on ALS disease progression, we crossbred SOD1 mice with mice depleted of the CGRP-specific receptor component receptor activity-modifying protein 1 (RAMP1), and monitored clinical and histological symptom development and progression under presence and absence of functional CGRP signaling. SOD1:RAMP1-/- mice showed an earlier onset of hind limb motor deficits compared to SOD1:RAMP1+/+ mice (56 ± 30 days vs. 92 ± 20 days), while overall survival was similar (median 142 days vs. 137 days). On the cellular level, motor neuron degeneration in the lumbar aspect of the spinal cord started earlier in SOD1:RAMP1-/- mice when compared to SOD1:RAMP1+/+ mice, but was followed by decelerated motor neuron loss throughout disease progression. In addition, morphological activations of astrocytes and microglia, and lymphocyte infiltration were attenuated in SOD1:RAMP1-/- mice, both in pre-symptomatic, early symptomatic, and end-stage when compared to SOD1:RAMP1+/+ mice.
The observed effects under suppression of CGRP signaling in SOD1 mice suggest in turn that the secretion of CGRP by motor neurons is neuroprotective predominantly at disease-onset, possibly through stimulation of a protective neuro-inflammatory milieu. In contrast, CGRP signaling on glia at later stages furthers disease progression by promoting chronic neurodestructive neuroinflammation.
References: Ringer C, Weihe E, Schütz B (2009) Neurobiol. Dis. 35:286-295; Ringer C, Weihe E, Schütz B (2012) Neurobiol. Dis. 45:547-554.
Acknowledgements: This study was supported by a research fund from the University Medical Center Giessen and Marburg (UKGM), the Foundation P.E. Kempkes Marburg, and the German Society for the Muscular Diseased (DGM).

Disclosures: C. Ringer: None. **K. Tsujikawa:** None. **E. Weihe:** None. **B. Schütz:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.27/N10

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Endothelin-1 is over-expressed in ALS and induces degeneration of cultured motor neurons

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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons (MNs) and astrogliosis. Several evidences suggest that the vulnerability of MNs is not cell-autonomous. Namely, factors secreted by activated astrocytes might contribute to MN degeneration and therefore their identification is important to develop new therapeutic strategies aimed at slowing disease progression and increasing survival of ALS patients. We focused on endothelin-1 (ET-1), a peptide which is strongly up-regulated in reactive astrocytes under different pathological conditions. ET-1 mRNA has been found to be up-regulated in the cortex of ALS patients. Furthermore, an increase in ET-1 levels in the CNS has been reported following spinal cord injury, a condition characterized by an extensive reactive gliosis.

We performed immunohistochemistry experiments in the G93A mouse model of ALS and in the spinal cord of ALS patients and revealed that ET-1 is strongly expressed in reactive astrocytes in both mouse and human specimens. To study a possible role of ET-1 on MN degeneration we used mixed spinal cord cultures enriched in reactive astrocytes and treated them with increasing concentrations of ET-1 for different length of time (8-72 hours). We chose an exposure of ET-1 (100-200 nM) for 48 hours, which resulted consistently in the death of 40-50% of MNs and 20% of other neuronal cells, whilst it did not exert any effect on astrocytes as revealed by immunocytochemistry and Western blotting analysis with SMI32, anti-MAP2 and anti-GFAP antibodies. ET-1 acts on both ET-A and ET-B receptors. ET-1 effect was mimicked by ET-3 (100 nM) and sarafotoxin S6C (10 nM), two selective agonists of ET-B receptors, and was not additive with that of ET-3 suggesting the involvement of ET-B receptors. Surprisingly, however, the ET-1 effect was only slightly reversed by the ET-A receptor antagonist BQ123 (2 uM), but was not affected by the ET-B receptor antagonist BQ788 (200 nM-2 uM), suggesting an atypical pharmacological profile of the receptors responsible for the observed ET-1 toxicity. ET-1 effect was not reversed by the ionotropic glutamate receptor AMPA antagonist GYKI 52466 (20 uM), suggesting that it is not caused by an increased glutamate release. Conversely, a 48 hour ET-1 treatment increased MN death induced by the acute exposure to AMPA (50 uM). Importantly, ET-1 did not induce MN death when administered on cell cultures treated with AraC (5 uM) or grown in a serum-free medium that did not favour astrocytes proliferation.

Overall these data suggest that ET-1 may contribute to MN degeneration in ALS through a mechanism mediated by reactive astrocytes.

Disclosures: E. Ranno: None. **M. Catania:** None. **S. D'Antoni:** None. **A. Berretta:** None. **F. Laureanti:** None. **M. Spatuzza:** None. **R. Pellitteri:** None. **P. Longone:** None. **A.M. Iyer:** None. **E. Aronica:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.28/N11

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CONACYT Mexico project 128229

DGAPA, UNAM project IN215610

Title: Chronic infusion of 4-aminopyridine in the spinal cord *In vivo* induces motor alterations but no neurodegeneration

Authors: R. LAZO-GÓMEZ, *R. TAPIA;
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Abstract: Amyotrophic lateral sclerosis is a devastating neurodegenerative disorder characterized by motor neuron (MN) death and paralysis. There is evidence that glutamate-mediated excitotoxicity may participate in MN death (Exp. Opin. Ther. Targets 11: 1415; 2007). On this base, we designed a model of chronic spinal MN neurodegeneration in vivo through the infusion of AMPA directly into the lumbar spinal cord of rats, using osmotic minipumps. Such chronic activation of AMPA receptors produces MN degeneration and paralysis of the hindlimbs 3-6 days after the beginning of AMPA infusion (J. Neuropathol. Exp. Neurol. 66: 913; 2007). We have now studied the effect of increasing endogenous glutamate by means of the infusion of 4-aminopyridine (4-AP), a K+-channel blocker that stimulates glutamate release. 4-AP [35 mM] was infused during 15 days, and motor changes were measured in the rotarod test and the paw grip endurance test (PGE).The number of MNs was assessed by histological observations and ChAT immunohistochemistry. One day after the beginning of 4-AP infusion the animals showed intermittent fasciculations in both hindlimbs and a significant decrease in the rotarod performance, but no alterations in the PGE test; these changes lasted 4-6 days and then gradually disappeared. However, quantitative histological observations showed that the number of healthy MN was normal. The motor alterations observed suggested MN hyperexcitation, possibly mediated by endogenous glutamate, so we tested if NMDA receptors could be involved by co-infusing MK-801 [1 mM] with 4-AP. In both motor tests, rats showed normal behavior, similar to that of control rats, and no significant changes were observed histologically. Since the neurotransmitter release induced by 4-AP is not specific for glutamate but may also affect GABA, which could be inhibiting the hyperexcitation and thus preventing MN death, we infused bicuculline [10 mM] (BIC) alone or in combination with 4AP. In the BIC + 4-AP group,

fasciculations were more frequent and intense, but the deficit in the rotarod test was similar to that after 4-AP alone, and again no MN degeneration was observed. On the other hand, BIC infusion alone produced compulsive scratching of the ipsilateral hindlimb and a significant loss of MN. We conclude that the hyperexcitation produced by 4-AP is probably due to NMDA receptor activation by the increased release of endogenous glutamate. This hyperexcitation was not enough for inducing MN death, due possibly to an increased inhibitory effect of GABAergic synapses.
This research was supported by CONACYT (project 128229) and DGPA, UNAM, project IN215610.

Disclosures: R. Lazo-Gómez: None. **R. Tapia:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.29/N12

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Different proteins aggregation and genes expression in mutated als patients

Authors: *C. CEREDA[1], P. MILANI[1], S. GAGLIARDI[1], O. PANSARASA[1], L. DIAMANTI[2], F. POLVERACCIO[2], S. LA SALVIA[2], L. DRUFUCA[2], M. CERONI[1,2];
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Abstract: Alterations in RNA metabolism have been shown in ALS opening new research perspectives on its pathogenesis (Strong 2010). In this context, we have already demonstrated the increase of SOD1 mRNA level in SALS patients tissues compared to controls (Gagliardi et al., 2010). Herein, we described SOD1, TARDBP and FUS mRNA levels and protein aggregation in PBMCs of mutated, non-mutated sporadic ALS patients (SALS) and sex- and age-matched healthy subjects. We analyzed 70 SALS and 70 controls for SOD1, TARDBP, FUS by Real Time PCR. We also included SALS patients mutated in SOD1 (L106F), TARDBP (A382T) and FUS (R521C).
We also performed immunofluorescence experiments to morphologically evaluate the sub-cellular distribution and appearance of the three proteins in lymphoblasts of mutated, non mutated patients and matched controls.
We confirmed that SOD1 mRNA level was up-regulated in ALS patients compared to controls and, interestingly, SOD1 expression in mutated patient was higher than in non-mutated patients.

We demonstrated that the higher level is determined by the mutated allele.
Immunofluorescence of lymphoblasts showed the presence of SOD1 cytoplasmatic inclusions in SOD1 mutated patient and sporadic cases. Small aggregates were also observed in patients mutated in FUS. About TARDBP expression, we showed that the mRNA level was similar between SALS and controls but we evidenced that the TARDBP expression in the mutated SALS patient was higher than controls and SALS non mutated. As SOD1, the higher mRNA level was regulated by the mutated allele. Large round-shaped cytoplasmatic speckles were evident in the patient with mutation in TDP-43, while smaller inclusions were present in sporadic cases.
Finally, we measured FUS mRNA level in SALS patients, in mutated cases and controls and no difference were detectable among these groups. Immunofluorescence experiments showed a diffuse distribution of FUS protein in the nuclear compartment of lymphoblasts from all ALS cases as well as control subjects. We demonstrated that genetic mutations can impact both gene expression and protein aggregation. In fact, mutations in SOD1 and TARDBP gene direct correlate with mRNA level and distribution of the protein aggregation.About FUS, we hypothesized that its involvement in ALS is not related to gene expression and protein aggregation, but, concerning the mutated patients, a distinct clinical phenotype seems to characterize cases carrying the R521C mutation (Corrado et al., 2009). Only samples with different FUS mutations may clarify these data. We will amplify our study by recruitment SALS patients with different mutations in SOD1, TARDBP and FUS genes to confirm our data.

Disclosures: C. Cereda: None. **P. Milani:** None. **S. Gagliardi:** None. **O. Pansarasa:** None. **L. Diamanti:** None. **F. Polveraccio:** None. **S. La Salvia:** None. **L. Drufuca:** None. **M. Ceroni:** None.

Poster

529. **Motor Neuron Disease: Mechanisms III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 529.30/N13

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: N-RENNT, VW Foundation/MWK Niedersachsen

Deutsche Muskelstiftung

Title: Regulation of the cytoskeleton in spinal muscular atrophy (sma)

Authors: N. HENSEL, B. FÖRTHMANN, H. BRINKMANN, *P. CLAUS;
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Abstract: SMA is a neurodegenerative disease caused by progressive degeneration of motoneurons in the spinal cord due to deletion or mutation of the survival of motoneuron (Smn1) gene. In our previous work we have analyzed how SMN regulates neurite growth in neuronal cells. Loss of SMN severely affects regulation of the actin cytoskeleton by dysregulation of the Rho-kinase (ROCK) pathway (van Bergeijk, 2007; Nölle et al., 2011). The SMN-binding protein profilin is the functional link between SMN and this pathway. SMN binds to profilin2 and this interaction becomes interrupted by SMN loss or due to the SMN point mutation S230L close to the profilin2 binding domain. As a consequence, more profilin2 binds to ROCK resulting in its hyper-phosphorylation by ROCK.
Which ROCK downstream molecules are responsible for axonal pathology and possible protection in SMA? In this study, we further dissected the molecular pathway by evaluating the individual roles of downstream targets in disease pathology. Since we have previously found dysregulations of LIMK/cofilin, profilin2a and MLCP at the level of phosphorylation in vitro and in vivo, we wanted to determine which of these targets affect motility-based processes and destabilize axons in SMA. Moreover, we analyzed the effects of activation and inhibition, respectively, of the ROCK pathway as well as ERK signalling, which is functionally linked to ROCK, on neurite outgrowth in an cellular SMA model.
ROCK is a central hub for signal integration and regulation of the actin cytoskeleton as well as actomyosin activitiy. Interestingly, ROCK inhibition by Y27632 or Fasudil improves the lifespan of SMA mice (Bowerman et al., 2010). ROCK inhibition could reverse the observed hyper-phosphorylation of its profilin downstream target. Remarkably, mutations of phosphorylation sites of profilin1 have recently been linked to the pathogenesis of the motoneuron disease amyotrophic lateral sclerosis (Wu et al., 2012). On the basis of these data, we hypothesize that cytoskeletal changes including both actin- and myosin-based processes could be relevant for disease pathogenesis in SMA.

Disclosures: N. Hensel: None. **B. Förthmann:** None. **H. Brinkmann:** None. **P. Claus:** None.**Poster**

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.01/N14

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: North Rhine Westphalia

SFB629 programm project grant from the German Research Council (DFG)

Title: Reduced protein translation rates in a *Drosophila* model for GARS-associated CMT peripheral neuropathy

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Abstract: Charcot-Marie-Tooth neuropathy (CMT) is characterized by selective degeneration of peripheral motor and sensory nerves, leading to progressive muscle weakness and wasting and sensory loss. The disease is both clinically and genetically heterogeneous, with currently more than 30 genes causally linked to CMT. Dominant mutations in at least three distinct tRNA synthetase genes cause CMT, namely glycyl-tRNA synthetase (GARS), tyrosyl-tRNA synthetase (YARS) and alanyl-tRNA synthetase (AARS). tRNA synthetases catalyze the aminoacylation of tRNAs with their cognate amino acid, and are therefore essential for protein synthesis.
The fact that dominant mutations in three tRNA synthetases cause CMT suggests that alteration of a common function of these enzymes could be the cause of disease, most probably the canonical aminoacylation activity. However, we and others have previously shown that loss of aminoacylation activity *per se* is not necessary to cause disease, as some CMT-associated mutations do not affect enzymatic activity. However, this does not exclude the possibility that CMT mutations could alter the subcellular localization of the mutant enzymes, what may lead to defects in local protein synthesis.
We here report the generation and characterization of a *Drosophila* model for GARS-associated CMT. A number of hallmarks of the human disease are recapitulated in our *Drosophila* model, including motor performance deficits, reduced life span, defects in neuromuscular junction morphology, and defects in sensory neuron morphology. Furthermore, we studied the subcellular localization of the mutant GARS proteins, but did not find any alterations, indicating that defects in local protein synthesis due to altered subcellular localization is unlikely. We nevertheless evaluated protein synthesis rates in motor neurons *in vivo*, using FUNCAT and BONCAT technology to label newly synthesized proteins. Unexpectedly, we found significantly reduced protein translation rates upon expression of two of the three mutant GARS transgenes. Possible underlying mechanisms will be discussed.
In conclusion, the use of a novel technology has allowed us to detect, for the first time, reduced global protein translation rates induced by a CMT-mutant tRNA synthetase. Our findings may provide important new insights into the molecular pathogenesis of CMT associated with mutations in tRNA synthetases.

Disclosures: E. Storkebaum: None. **S. Niehues:** None. **J. Bussmann:** None. **G. Steffes:** None. **I. Erdmann:** None. **S. Koerdt:** None. **M. Lysaja:** None. **D. Dieterich:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.02/N15

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: HHMI

NIH

Pioneer Fund

Title: Novel mechanistic insight into the pathology of hereditary motor and sensory neuropathy

Authors: *G. BAI[1], W. HE[2], H. LIU[3], H. ZHOU[2], N. WHITE[1], V. I. SHUBAYEV[3], R. W. BURGESS[4], X.-L. YANG[2], S. L. PFAFF[1];
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Abstract: Charcot-Marie-Tooth (CMT) disease is the most common hereditary peripheral neuropathy for which there is no effective therapy. CMT type 2D is caused by dominant mutations in *GARS* gene, encoding glycine tRNA synthetase (GlyRS). Despite the ubiquitous requirement for this cytoplasmic enzyme in protein synthesis, mutations in GlyRS cause selective peripheral axon degeneration leading to deficits in distal motor function. Previous genetic studies indicate that CMT2D is caused by a neomorphic function of GlyRS mutants (i.e. gain-of-function), but the underlying molecular mechanism is still not known. Our biochemical and cellular data has unmasked a novel non-canonical biological pathway in which mutant GlyRS is secreted and binds competitively with a neurotropic factor to its receptor. Importantly, this mutant-specific interaction interferes with the neurotropic pathway, controlling the progression of the neuropathy in a mouse model of CMT2D. These studies lead to a paradigm shift in our understanding of the function of GlyRS in CMT2D pathogenesis, shedding light on innovative treatment for this disease.

Disclosures: G. Bai: None. **N. White:** None. **W. He:** None. **X. Yang:** None. **H. Zhou:** None. **R.W. Burgess:** None. **H. Liu:** None. **V.I. Shubayev:** None. **S.L. Pfaff:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.03/N16

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: the Ministry of Health, Labour and Welfare Grant

the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant

the Japan Society for the Promotion of Science (JSPS) Grant

Title: Modeling the early phenotype at the neuromuscular junction of spinal muscular atrophy using patient-derived iPSCs

Authors: *M. YOSHIDA, S. KITAOKA, N. EGAWA, M. YAMANE, K. TSUKITA, T. NAKAHATA, H. INOUE, M. SAITO;
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Abstract: Objective: To establish an in vitro model of neuromuscular junction (NMJ) connectivity using motor neurons differentiated from spinal muscular atrophy (SMA) patient-derived induced pluripotent stem cells (iPSCs) in order to evaluate the major factors contributing to the pathogenesis of SMA.
Background: SMA is a neuromuscular disorder caused by mutations of the survival of motor neuron 1 (SMN1) gene. It remains unclear why the reduction of the ubiquitously expressed SMN protein selectively affects the neuromuscular system in SMA patients. Recent findings suggest that impaired NMJ formation is an important hallmark in SMA. However, the contribution of NMJ pathology to the pathogenesis of SMA remains unclear due to the limited availability of tissue samples.
Design/Methods: We established human iPSCs from SMA patients and healthy control subjects. The iPSCs were differentiated into motor neurons, and were co-cultured with mouse myotubes to form NMJ *in vitro*. The number of motor neurons and the mean area of acetylcholine receptor (AChR) clustering were evaluated by immunocytochemistry.
Results: While AChR clustering was successfully established on myotubes co-cultured with control iPSC-derived motor neurons, this was significantly impaired in those cultured with SMA iPSC-derived motor neurons. On the other hand, no significant neuronal death was observed in the SMA iPSC-derived motor neurons compared to those from healthy controls.
Conclusions: We successfully developed an *in vitro* model of NMJ,
and by using SMA-iPSCs, we demonstrated that low levels of SMN in the motor neurons are responsible for the impairment of AChR clustering. Our findings imply that the loss of neuromuscular connections appears prior to motor neuronal death, thus suggesting that the NMJ defect is likely to be a major contributing factor to the pathogenesis of SMA. The current *in vitro* NMJ model is useful to dissect the pathophysiological mechanisms underlying the development

of SMA, and to evaluate the efficacy of new therapeutic approaches.

Disclosures: M. Yoshida: None. **S. kitaoka:** None. **N. egawa:** None. **M. yamane:** None. **K. tsukita:** None. **T. nakahata:** None. **H. inoue:** None. **M. saito:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.04/N17

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01NS064224

MDA Grant (MDA4209)

Families of SMA

Title: Function of ZPR1 in neurodegeneration and pathogenesis of SMA

Authors: *L. D. GANGWANI[1], N. GENABAI[1], S. AHMAD[2];
[1]Biomed. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., El Paso, TX; [2]Georgia Regents Univ., Augusta, GA

Abstract: Background: Spinal muscular atrophy (SMA) is caused by mutations of the *Survival Motor Neurons* (*SMN1*) gene. SMA is characterized by degeneration of spinal motor neurons caused by low levels of SMN protein. The copy number of the *SMN2* gene primarily influences the severity of SMA. Additional modifier genes that lie outside the SMA locus exist and one gene that could modify SMA is the *Zinc Finger Protein* (*ZPR1*) gene. Currently, there is no treatment available to cure or reduce the burden of severity of SMA because of limited knowledge of modifier genes and the molecular mechanisms associated with SMA pathogenesis.
Objective: To examine the role of ZPR1 in spinal cord motor neuron degeneration and pathogenesis of SMA.
Methods: *In vivo* studies using *Zpr1* knockout and SMA model mice. *In vitro* studies using cultured primary spinal cord neurons.
Results and Discussion: To test the significance of ZPR1 down-regulation in SMA, we examined the effect of reduced ZPR1 expression in mice with mild and severe SMA. We report that the reduced ZPR1 expression causes increase in the loss of motor neurons, hypermyelination in phrenic nerves, increase in respiratory distress and disease severity that reduces lifespan of

SMA mice. The deficiency of SMN-containing sub-nuclear bodies correlates with the severity of SMA. ZPR1 is required for accumulation of SMN in sub-nuclear bodies. We report that ZPR1 overexpression increases levels of SMN and promotes accumulation of SMN in sub-nuclear bodies in SMA patient fibroblasts. ZPR1 stimulates neurite growth and rescues axonal growth defects in SMN-deficient spinal cord neurons from SMA mice. These data suggest that the severity of disease correlates negatively with ZPR1 levels and ZPR1 may be a protective modifier of SMA.
Conclusions: ZPR1 deficiency causes defects in phrenic nerve that may contribute to respiratory distress and increase severity of SMA. Because SMA patients express low levels of ZPR1, our data suggest phrenic nerve as a potential therapeutic target to reduce the burden of respiratory distress in SMA. ZPR1 overexpression elevates SMN levels, corrects the defect in nuclear accumulation of SMN in SMA patient cells and rescues the axonal growth of SMN-deficient neurons from SMA mice. These findings suggest that ZPR1 may be a protective modifier of SMA and opens new avenues for SMA therapeutics.
Acknowledgements: Funding from the Muscular Dystrophy Association, Families of SMA and NIH (R01NS064224) to LG supported this study.

Disclosures: L.D. Gangwani: None. **N. Genabai:** None. **S. Ahmad:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.05/N18

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH RO1 AR046799

NIH PO1 NS058901

NIH RO1 AG037984

NIH R37 AG036800

Evelyn F McKnight Brain Research Foundation

Title: Mouse muscleblind-like compound knockout models of myotonic dystrophy

Authors: *K.-Y. LEE[1,2,4], M. LI[1,2], M. MANCHANDA[1,2], D. FINN[1,2], A. KUMAR[1,3], T. FOSTER[1,3], M. SWANSON[1,2];

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Abstract: The muscleblind-like (MBNL) proteins are RNA binding factors that regulate alternative splicing during development by promoting the production of adult protein isoforms. The *MBNL* family consists of three genes, *MBNL1*-3, and we have proposed that sequestration of all three MBNL proteins by the toxic C(CUG) repeat expansion RNAs expressed from mutant *DMPK* and *CNBP* genes causes the multisystemic disease myotonic dystrophy (dystrophia myotonica, DM). To test this hypothesis, we have previously reported the generation of *Mbnl1*, *Mbnl2* and *Mbnl3* knockout (KO) mice. Although Mbnl1 and 2 proteins are expressed in the majority of adult somatic tissues and recognize RNAs via a similar YGCY binding motif, *Mbnl1* KO mice recapitulate several skeletal muscle features of DM while *Mbnl2* KO mice are a good model for DM-relevant CNS disease. To test if loss of both Mbnl1 and Mbnl2 resulted in additional skeletal and cardiac muscle phenotypes associated with DM, we generated *Mbnl1*; *Mbnl2* compound KO mice. While *Mbnl1*$^{-/-}$; *Mbnl2*$^{-/-}$ double KOs are embryonic lethal, *Mbnl1*$^{-/-}$; *Mbnl2*$^{+/-}$ KOs are viable but have a limited lifespan and develop skeletal muscle weakness/wasting as well as heart conduction block, which are cardinal features of DM missing in *Mbnl1* and *Mbnl2* single KOs. These additional DM phenotypes likely result from an increase in defective splicing regulation and the nearly complete reversal to fetal isoform expression in adults that results from depletion of Mbnl2 in the *Mbnl1* KO background. This study supports the MBNL loss-of-function hypothesis for DM and provides additional genetic models for this RNA-mediated disease.

Disclosures: K. Lee: None. **M. Li:** None. **M. Manchanda:** None. **A. Kumar:** None. **D. Finn:** None. **T. Foster:** None. **M. Swanson:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.06/O1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Longitudinal neuromuscular responses in mdx mice challenged with or without addition of forced exercise

Authors: *A. E. KUDWA, Y. JIMENEZ, D. GOMEZ, A. SANCHEZ, R. STEVENSON, B. ALOSIO, R. MUSHLIN, K. CIRILLO, S. RAMBOZ;
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Abstract: Duchenne muscular dystrophy (DMD) is a lethal X-linked neuromuscular disease caused by a mutation in the dystrophin gene, and the *mdx* mouse is the most widely used model of DMD used in preclinical therapeutic screening. Many neuromuscular diseases such as DMD are characterized by an exaggerated exercise-induced fatigue response, disproportionate to activity level, known as fatigability which is observed in both DMD patients and the *mdx* mouse. As such, we developed a hind limb fatigue challenge (HLFC) test utilizing postural fatigue and identified a robust, early *in vivo* fatigability phenotype in the *mdx* mouse. In the HLFC test, subjects are evaluated in a locomotor task, are then forced to stand on their hind limbs for an extended period of time, and are subsequently evaluated in the same locomotor task. Whereas other exercise paradigms such as the running wheel or treadmill can be used to elicit whole body fatigue incorporating more robust cardiopulmonary components, the HLFC test isolates the fatigue to the hindlimb muscles, and we observe consistent early differences in fatigability between *mdx* and control animals. However, as the *mdx* mouse exhibits substantial muscle regeneration leading to a mild progression of the disease, the extent of muscle pathology in sedentary adult mdx mice is low and running wheel or treadmill exercise are often used to exacerbate myofibre necrosis, biomarkers indicative of muscle damage, and behavioral deficits. Therefore, the current study directly compares cohorts of mice evaluated in the HLFC longitudinally with or without exercise in between HLFC testing ages. Ultimately, our goal is to develop a behavioral battery which will reliably capture neuromuscular deficits and provide for efficient and robust preclinical testing in the *mdx* mouse model of DMD. The effectiveness of currently used compounds with therapeutic value in DMD patients, such as prednisone, will be screened using the newly established efficacy study platform.

Disclosures: A.E. Kudwa: None. **Y. Jimenez:** None. **D. Gomez:** None. **A. Sanchez:** None. **R. Stevenson:** None. **B. Alosio:** None. **R. Mushlin:** None. **K. Cirillo:** None. **S. Ramboz:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.07/O2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Inhibitor of hematopoietic prostaglandin D synthase improves the muscle disorder in an experimental model of Duchenne muscular dystrophy

Authors: K. TANAKA[1,2], K. ARITAKE[2], K. MIYOSHI[1], *Y. HAYASHI[1], E. SASAKI[1], Y. URADE[2];

[1]Taiho Pharmaceut. Co., Ltd., Tsukuba, Japan; [2]Dept. of Mol. Behavioral Biol., Osaka Biosci. Inst., Suita, Japan

Abstract: Duchenne muscular dystrophy (DMD) is a fatal genetic disease. The fibers of muscle in DMD patients easily suffer necrotic change, because of the loss of dystrophin that is a membrane protein which links the intracellular cytoskeleton to the extracellular matrix. Thus the patients present with a weakening motor activity. There is still no complete cure for the disease. However, we recently reported that prostaglandin D_2 (PGD_2) synthesized by hematopoietic prostaglandin D synthase (HPGDS) may play an important role in the pathology of DMD, especially muscle necrosis. In this study, to determine the beneficial effect of HPGDS inhibition in therapy for DMD, we evaluated the effect of a highly selective inhibitor, which is a 2-phenoxypyrimidine-5-carboxamide derivative (TFC-007) found in our laboratory, in dystrophine-deficient mice.

We used Dystrophin-deficient *mdx* mice (C57BL/6 back ground) and the wild-type mice (C57BL/6). Mice were orally administrated TFC-007 (30 mg/kg/day) or vehicle for 4 weeks from 4 to 8 weeks of age. At 8 weeks of age, we measured the locomotor activity during night-time, collected the mouse urine of at night-time to determine the urinary concentration of tetranor-PGDM, a metabolite of PGD_2, evaluated the volume of necrotic muscle by measuring Evans blue dye leaked to the skeletal muscle after intravenous injection of the dye, and counted the number of necrotic fibers in cross sections of the tibialis anterior muscle (TA).

The locomotor activity at the night-time was significantly lower in *mdx* mice than that in wild-type mice. The dye-leakage was enhanced in *mdx* mice as compared with the wild-type animals. In histological evaluation, many necrotic fibers were detected in TA of *mdx* mice but hardly in wild-type mice. The urinary tetranor-PGDM concentration was significantly higher in *mdx* mice than that in wild-type mice. TFC-007 administration to *mdx* mice significantly suppressed the urinary tetranor-PGDM concentration, reduced both the dye-leakage and the necrotic muscle fibers, and recovered the locomotor activity.

These results indicate that PGD_2 synthesized by HPGDS is involved in the progression of muscular necrosis in DMD and the inhibition of HPGDS would be an effective therapy for DMD.

Disclosures: K. Tanaka: A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., ltd.. **K. Aritake:** None. **K. Miyoshi:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., ltd. **Y. Hayashi:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., ltd. **E. Sasaki:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., ltd.. **Y. Urade:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.08/O3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH R15AR055360

Title: The effects of Ursodeoxycholic Acid (UDCA) on the function of isolated mdx gastrocnemius and costal diaphragm preparations

Authors: *C. CARLSON[1], D. LUO[2], H. YU[2], R. POTTER[2];
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Abstract: Previous studies using the mdx model for Duchenne muscular dystrophy indicated that daily *in vivo* administration of the NF-kappaB inhibitor, ursodeoxycholic acid (UDCA), significantly increased the whole body tension (WBT), a noninvasive behavioral measure of the forward pulling tension exerted by the limb musculature. The purpose of the present studies was to assess the effects of this treatment on isolated muscle function. One month old mdx mice were treated with daily intraperitoneal (ip) injections of UDCA (40 mg/kg) or vehicle solution (1.02 % NaCl, pH 8.4) for a period of 1 month. At the end of this treatment period, the mice were anesthetized with isoflurane and the gastrocnemius-plantaris muscle preparation was isolated and used for determinations of active isometric tension using direct stimulation *in situ*. In addition, the mice were euthanized and costal diaphragm strips were used to determine active isometric tension with direct stimulation *in vitro*. Initial results indicate that, at 2 months of age, the mdx gastrocnemius-plantaris preparation does not exhibit alterations in optimal length (l_o) or deficits in specific twitch or tetanic tension compared to age-matched nondystrophic preparations. UDCA treatment did not influence any of these muscle function parameters in the mdx gastrocnemius-plantaris preparation. In contrast, the costal diaphragm of vehicle treated mdx mice exhibited a significant reduction in l_0 ($p<0.001$) and specific twitch ($p<0.01$) compared to nondystrophic preparations. Initial results indicate that UDCA treatment significantly increased l_o ($p<0.05$) and significantly increased the specific tetanic tension at 75 ($p<0.05$) and 100 Hz ($p<0.05$). UDCA - induced increases in specific twitch and specific tetanic tension at 25 and 50 Hz just failed to reach significance ($0.05 < p < 0.08$). These results suggest that mdx mice exhibit a deficit in specific tension in the diaphragm primarily because of decreases in l_o that are presumably secondary to sarcomere disorganization. The partial reversal of this deficit by treatment with UDCA and other NF-kappaB inhibitors may indicate partial restorative effects on sarcomere organization in dystrophic muscle.

Disclosures: C. Carlson: None. **D. Luo:** None. **H. Yu:** None. **R. Potter:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.09/O4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 NS058901

NIH P01 NS058901

NIH P30 NS062158

Title: Autofluorescence optical imaging of the responses to intracortical stimulation in normal mouse models of myotonic dystrophy

Authors: *G. CHEN[1], S. W. CRAMER[1], L. P. W. RANUM[2], M. S. SWANSON[2], T. J. EBNER[1];
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Abstract: Patients with myotonic dystrophy (DM) have cognitive as well as motor function deficits with widespread changes in cerebral cortex white matter. In DM1, CUG, and in DM2 CCUG, expansion transcripts sequester muscleblind-like (MBNL) proteins leading to alternative splicing abnormalities that contribute to disease so restoring MBNL activity is a possible therapeutic strategy. In this study, we use autofluorescence imaging, pharmacological and electrophysio-logical techniques to assess cerebral cortical circuit function in vivo in normal and transgenic mouse models of DM.
In normal anesthetized FVB mice, the cerebral cortex is exposed bilaterally and intracortical microstimulation (ICMS) is delivered with a tungsten microelectrode. ICMS with a single pulse in the motor cortical region evokes a center surround inhibitory response in both hemispheres. The surround decrease in fluorescence is completely blocked by bath application of a GABAA antagonist (SR95531), whereas the central increase in fluorescence is greatly enhanced. Train stimulation (10 Hz, 1s) evokes a strong increase in fluorescence in the stimulated hemisphere centered on the electrode and a "mirror" increase in the contralateral hemisphere that are mediated post-synaptically by AMPA and NMDA glutamatergic receptors. The response in the contralateral cortex is mediated by the corpus callosum as it is blocked by microinjection of the neuro-toxin TTX into the corpus callosum with no significant changes ipsilaterally. Blocking nitric ox-ide synthase (with L-NAME) or cyclooxygenase (with indomethacin), major mediators of in-creased blood flow in response to neural activity, has no effect on the responses. Together, these findings demonstrate the neural and post-synaptic origin of these evoked responses.
In Mbnl2 knockout mice, the response to 10 Hz ICMS train stimulation is prolonged and larger regions are activated in both hemispheres of the cerebral cortex. In mice that overexpress

MBNL1 (MBNL1-OE), the inhibitory surround signal evoked by a single pulse stimulus is markedly reduced with a significant increase in the center compared to wild-type mice. The response in the contralateral hemisphere evoked by 10 Hz stimulation is reduced in MBNL1-OE versus wild-type mice. However, the response to ICMS in the ipsilateral cortex is normal. Therefore, altered MBNL1 expression modifies cerebral cortical excitability, including the responses mediated by the corpus callosum and these changes may contribute to the cognitive disorders in DM patients. Further, our results demonstrate that flavoprotein imaging is a useful tool to assess cere-bral cortical function in mouse models of DM.

Disclosures: G. Chen: None. **S.W. Cramer:** None. **L.P.W. Ranum:** None. **M.S. Swanson:** None. **T.J. Ebner:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.10/O5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Indian Council of Medical Research,New Delhi, INDIA N0.3/13/JRF-2012/HRD

Title: Neuropsychological assessment of dystrophin isoform induced progression of cognitive impairment in DMD/BMD patients in North West Indian Population

Authors: *R. TYAGI, S. PRABHAKAR, A. ANAND;
Dept. of Neurology, Post Grad. Inst. of Med. Educ. and Resear, Chandigarh, India

Abstract: Duchenne muscular dystrophy [DMD] and Becker's Muscular Dystrophy [BMD] are among the most common type of muscular dystrophy with X-linked inheritance. In DMD patients respiratory failure and cardiomyopathy are the cause of death in their twenties. Progression of BMD is less severe than DMD with late age of onset. Dystrophin isoforms DP140, DP71, DP40, which are predominantly expressed in brain from distinct internal promotors, are known to be associated with cognitive decline. DP71 plays a role in synaptic transmission and plasticity through clustering glutamate receptor by interacting with multi scaffolding proteins. DP40 isoform interacts with presynaptic proteins which plays role in exocytosis. DP140 expresses predominantly during fetal brain development. One third of boys affected with DMD manifest cognitive impairment with mean Intelligence Quotient below 1.0-1.5 SD caused due to mutation in dystrophin gene. Complete analysis of dystrophin gene for deletion and duplication was carried out for patients with clinical features of Duchenne and Becker's Muscular Dystrophy in north-west Indian population using Multiplex Ligation

dependent Probe Amplification (MLPA). Out of 20 patients analysed through MLPA in Neuroscience Research Lab, deletion was detected in 70 % patients. Out of these patients 78 % patients showed mutation in DP140 specific region. Neuropsychological assessment with analysis of sociodemographic factors for these patients would provide a clear evidence of dystrophin isoform specific cognitive deterioration and progression in follow up studies.

Disclosures: R. Tyagi: None. **S. Prabhakar:** None. **A. Anand:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.11/O6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Philanthropic Donations

Title: Changes in miRNA expression levels over disease course in a mouse model of ALS

Authors: *A. COURTRIGHT, S. VILLA, K. BURGOS, R. METPALLY, S. NASSAR, B. RAKELA, K. VAN KEUREN-JENSEN;
T-Gen, Phoenix, AZ

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a terrifying, fast moving and fatal disease with no treatment. Early therapeutic intervention could change this predicament by creating the potential to slow down disease pathogenesis, or stop it altogether. Finding effective therapeutics for ALS and other neurodegenerative diseases has been hindered, partly because of the inability to diagnose the disease early before significant neuronal loss has occurred, and partly because we do not fully understand the underlying causes of the disease. miRNA expression levels are responsive to alterations in cellular processes, they reflect mechanistic changes that occur in cells/neurons as they are affected by disease, and therefore we could learn a great deal about the changes in cell function that accompany ALS by studying miRNA expression patterns. miRNAs have been found to play a role in most cellular processes and can have very specific temporal, spatial and cell specific expression. Abnormal expression of miRNAs has been detected in association with the cellular dysfunction in neurological diseases, including Multiple Sclerosis, Alzheimer's disease and Parkinson's disease. These studies have even pinpointed alterations in the expression of miRNAs that target key proteins known to be involved in the pathogenesis of disease. Using human subjects we are unable to determine the earliest time point (before symptom onset and diagnosis) that miRNA deregulation can be detected or which tissues are most sensitive to disease-related changes in miRNA expression. In order to address this lack of

data, we used the SOD1G93A mouse model of ALS to examine changing miRNA profiles over key time points in these animals.

Disclosures: A. Courtright: None. **S. Villa:** None. **K. Burgos:** None. **R. Metpally:** None. **S. Nassar:** None. **B. Rakela:** None. **K. Van Keuren-Jensen:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.12/O7

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NINDS R01NS062766

VA Merit Review Grant

Title: Dysregulation of Rac or Rho induces death of motor neurons and activation of these GTPases is altered in the G93A mutant SOD1 mouse model of ALS

Authors: *T. R. STANKIEWICZ[1,2], R. J. BOUCHARD[2], D. A. LINSEMAN[1,2];
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Abstract: Numerous studies have demonstrated a critical function for Rho GTPase family members (i.e., Rac, Rho, Cdc42) in neuronal development and survival. Although a pro-survival function for Rac has been reported in several neuronal cell types including motor neurons, the antagonistic relationship between Rac and Rho/Rho kinase (ROCK) signaling in neuronal survival remains poorly understood. In the current study, we examined the effects of targeted inhibition of Rac GTPase on motor neuron survival. Treatment with NSC23766, a selective inhibitor of the Rac-specific guanine nucleotide exchange factors, Tiam1 and Trio, induces death of embryonic stem cell-derived motor neurons. Following inhibition of Rac, motor neurons displayed activation of caspase-3, dephosphorylation of AKT and ERK5, and nuclear translocation of the BH3-only protein Bad. We also examined the effects of a constitutive activator of Rho, CN03, on motor neuron survival in vitro. In a manner similar to inhibition of Rac, constitutive activation of Rho induced a marked loss of neurites and significant cell death. Interestingly, inclusion of the ROCK inhibitor, Y-26732, partially protected motor neurons from either selective inhibition of Rac or constitutive activation of Rho. These data suggest that the balance between Rac and Rho signaling is critical for motor neuron survival. In accordance with this concept, in the G93A mutant Cu,Zn-superoxide dismutase (SOD1) mouse model of

amyotrophic lateral sclerosis (ALS), active Rac1-GTP immunoreactivity was markedly decreased in choline acetyltransferase (ChAT)-positive motor neurons within the lumbar spinal cord of end-stage mutant mice when compared to age-matched wild type littermates. In addition, although immunoreactivity for total RhoB localized principally to nuclei of ChAT-positive motor neurons from wild type mice, RhoB showed a pronounced redistribution to motor neuronal processes in end-stage mice harboring the G93A SOD1 mutation. Collectively, our data demonstrate that Rac and Rho are critical regulators of motor neuron survival and as a result, disruptions in the balance of their activities may contribute to the etiology of motor neurodegenerative diseases such as ALS. (Supported by a Merit Review award from the Department of Veterans Affairs to DAL)

Disclosures: T.R. Stankiewicz: None. **R.J. Bouchard:** None. **D.A. Linseman:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.13/O8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Detailed examination of microvesicle protein and whole transcriptome RNA content, including non-coding RNAs (miRNA, lncRNA), from IPS cell lines derived from patients with Amyotrophic Lateral Sclerosis and healthy controls

Authors: *L. GHAFFARI[1], B. HJELM[1], A. JAVAHERIAN[2], M. BURKHARDT[2], C. RAMOS[2], B. RAKELA[1], A. COURTRIGHT[1], M. ROSENOW[1], K. PETRITIS[1], W. TEMBE[1], R. METPALLY[1], K. VAN-KEUREN JENSEN[1];
[1]Tgen, Phoenix, AZ; [2]iPierian, Inc, San Francisco, CA

Abstract: Little is currently known about the contents of microvesicles that are released from cell populations of the central nervous system or their potential importance in health and disease. We are particularly interested in the differences in the cargo contained within microvesicles released from affected cells in patients with the neurodegenerative disease, Amyotrophic Lateral Sclerosis (ALS) and healthy control subjects. We created Induced Pluripotent Stem (IPS) cell lines from six patients with ALS and two healthy control subjects. The pluripotent stem cells were differentiated into motor neuron cultures. Four of the ALS patients had sporadic forms of the disease, one patient had a TDP-43 mutation, and one patient had a FUS mutation. We used ultracentrifugation to isolate the microvesicles that were released by the cultures into the media. We then used next generation sequencing to characterize the RNA contents of the microvesicles

(including small RNAs) and tandem mass tags for mass spectrometry quantification and identification of protein contents. We report our findings from these experiments.

Disclosures: L. Ghaffari: None. **B. Hjelm:** None. **A. Javaherian:** None. **M. Burkhardt:** None. **C. Ramos:** None. **B. Rakela:** None. **A. Courtright:** None. **M. Rosenow:** None. **K. Petritis:** None. **W. Tembe:** None. **R. Metpally:** None. **K. Van-Keuren Jensen:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.14/O9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Pape Adams Foundation

Philanthropic Donation

Title: Targeting a novel gene in amyotrophic lateral sclerosis

Authors: *B. TERZIC[1], B. RAKELA[1], S. VILLA[1], R. BOWSER[2], T. BEACH[3], A. COURTRIGHT[1], R. METPALLY[1], K. VAN-KEUREN JENSEN[1];
[1]Translational Genomics Res. Inst. (tgen), Phoenix, AZ; [2]Barrow Neurolog. Institute, St. Joseph's Hosp., Phoenix, AZ; [3]Banner Sun Hlth. Res. Inst., Sun City, AZ

Abstract: Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease, is a devastating illness that causes the degeneration of both upper and lower motor neurons (MN), leading to eventual muscle atrophy. ALS rapidly progresses into paralysis, with patients typically dying within three to five years from the onset of their symptoms (most commonly due to respiratory complications). Even after many years of research and drug trials, there is still no cure, and current therapies only succeed in increasing life-span by approximately three months. In a genome-wide association study conducted by the Translational Genomics Research Institute (TGen), a single-nucleotide polymorphism (SNP) in a novel gene was found to significantly alter risk for ALS. This novel protein exhibits altered levels of protein and mRNA expression throughout ALS disease progression in human subjects and animal models. We previously showed a significant up-regulation of this novel protein’s expression in muscle during end-stages of ALS disease progression in the SOD1G93A mouse model. Recent data from our lab showed a similar trend in human ALS muscle tissue. Mass spectroscopy analysis of proteins pulled down with this novel protein have identified proteins associated with RNA regulation, including gemin and other components of the SMN complex, as well as hnRNPs. To further validate these

findings, we have verified colocalization of these proteins with one another. We hypothesize that this protein plays an important role in ALS pathogenesis, and we will continue to examine its biological function.

Disclosures: B. Terzic: None. **B. Rakela:** None. **R. Bowser:** None. **T. Beach:** None. **A. Courtright:** None. **R. Metpally:** None. **K. Van-Keuren Jensen:** None. **S. Villa:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.15/O10

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Innervation-sensitive FGF-22 and FGFBP1 are necessary to maintain neuromuscular connections

Authors: *M. J. TENGA[1], H. UMEMORI[2], G. VALDEZ[1,3];
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Abstract: The neuromuscular junction (NMJ) undergoes deleterious structural changes before the erosion of motor skills during normal aging and progression of neurodegenerative diseases. This suggests that molecules that function to maintain and repair the NMJ could protect the motor system from a variety of insults. Here, we provide evidence indicating that the muscle-derived factors, fibroblast growth factor (FGF)-22 and the fibroblast growth factor binding protein 1 (FGFBP1), function to prevent the destruction of adult NMJs. In denervated skeletal muscles, there is a significant reduction in expression of both molecules. FGF-22 and FGFBP1 levels are also reduced in hind limb muscles of the SOD1-G93A mouse model for amyotrophic lateral sclerosis (ALS). In addition, there is a significant reduction in FGF-22 levels in aged, 24 month old, skeletal muscles. These results suggested that FGF-22 and FGFBP1 expressions could be under the control of nerve-derived factors. To test this idea, we treated cultured myotubes with the nerve-derived factor, z-agrin. This molecule is required to form and maintain the postsynaptic apparatus in vivo. It is also sufficient to induce the formation the postsynaptic apparatus in cultured myotubes. Z-agrin treatment of cultured myotubes results in a rapid and long-lasting upregulation of FGF-22 but not FGFBP1. To determine the role of FGF-22 in adult NMJs, we examined NMJs in aging FGF-22 null mice. In mice lacking FGF-22, there is an increased incidence of age-related alterations that include augmented postsynaptic fragmentation, partial denervation of muscle fibers, and extrasynaptic vesicle accumulation. Altogether, our data

suggests that FGF-22 and FGFBP1 may play roles in maintaining NMJs during normal aging, and potentially in diseases that affect the neuromuscular junction. These molecules could, therefore, hold therapeutic potential for preserving and reestablishing function of aged, injured, and diseases-affected NMJs.

Disclosures: M.J. Tenga: None. **H. Umemori:** None. **G. Valdez:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.16/O11

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Role of sensory neurons in the initiation and progression of Amyotrophic Lateral Sclerosis

Authors: *S. VAUGHAN, M. TENGA, Z. KEMP, G. VALDEZ;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is recognized as a progressive neurodegenerative disease that primarily targets motor neurons, which form neuromuscular junctions (NMJs) with skeletal muscles. The disease causes deleterious changes at the axon terminals of these neurons, leading to denervation of skeletal muscles, axonal degeneration, and ultimately death. Recent findings, however, indicate that ALS affects other neuronal populations in the spinal cord and brain. In this study, we sought to determine the involvement of sensory neurons in the initiation and progression of ALS using the SOD-G93A mouse model for the disease. To start, we examined changes in proprioceptive sensory neurons nerve endings (1a afferents) at muscle spindles. This strategy allowed us to compare pathological changes in sensory and motor neurons innervating the same muscles. We visualized proprioceptive sensory nerve endings and motor axon terminals in the extensor digitorum longus and gracilis muscles using antibodies against synaptotagmin-2 and neurofilament. In animals showing mild and severe ALS symptoms, the incidence of degenerating axons is similar between proprioceptive sensory neurons and motor neurons, irrespective of the muscle examined. We then visualized sensory synapses in the ventral horn of the spinal cord, primarily those formed with motor neurons. As in muscle spindles, there is a significant decrease in the number of vesicular glutamate transporter 1 (VGLUT1) positive synapses in the ventral horn of ALS-afflicted spinal cords. Interestingly, we found an increase in the number of parvalbumin-positive synapses, suggesting that proprioceptive axons sprout in the spinal cord potentially to compensate for loss of other synaptic inputs on motor neurons. These synaptic changes, along with changes in the

soma of sensory neurons, strongly suggest a key role for sensory neurons in ALS. It also demonstrates that the location of proprioceptive axons determines their response to ALS. These findings indicate that an effective therapeutic for ALS must also be designed to protect sensory neurons.

Disclosures: S. Vaughan: None. **M. Tenga:** None. **Z. Kemp:** None. **G. Valdez:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.17/O12

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH

DOD

Robert Packard Center for ALS research

ALSA

Title: Oligodendroglia significantly contribute to neuronal injury in ALS

Authors: *Y. LI[1], D. BERGLES[2], J. D. ROTHSTEIN[3];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by the loss of upper and lower motor neurons along with interneurons with unknown etiology. Recently we have amassed data suggesting that oligodendroglia contribute to ALS pathogenesis. Proliferating and reactive oligodendrocyte progenitor (OPCs, also known as NG2+ glia) are present in the lumbar spinal cord ventral horn gray matter where motor neurons die in a SOD1 ALS mouse model. We recently reported that oligodendroglia degenerate during disease development leading to loss of gray matter myelin. Changes in oligodendroglia, NG2 cells and myelin were also seen in human ALS motor cortex and spinal cord. Furthermore, oligodendroglial specific monocarboxylate transporter 1 (MCT1) expression is decreased in ALS mice and human ALS CNS. Given that oligodendrocytes not only facilitate saltatory conduction of action potentials by myelinating axons, but also support neuronal functions metabolically through MCT1, the injury to oligodendroglia in ALS may have pathogenic consequences. Therefore, to further investigate the role of oligodendrocytes in ALS pathogenesis, we selectively removed mutant hSOD1

(G37R) from oligodendrocyte progenitors in *PDGFαR-CreER;loxSOD1(G37R)* mice. The 4-hydorxytamoxifen (4HT) treatment caused significant decrease in the mutant human SOD1 transgene expression in OPCs. We found that excision of mutant hSOD1 from OPCs dramatically delayed disease onset and early disease, and significantly prolonged animal survival. In addition, at disease onset stage, activated astroglial and microglial responses were delayed in the animals received 4HT treatment. Moreover, the removal of mutant hSOD1 helped to preserve MCT1 expression at disease onset stage. These data indicate that expression of mutant hSOD1 in OPCs and their oligodendrocyte progeny has a deleterious effect on motor neuron survival, and suggest that a key negative consequence of mutant SOD1 expression in oligodendrocytes is to diminish their capacity to provide metabolic support to neurons. To further investigate the mechanisms involved in oligodendrocyte degeneration in human ALS, we differentiated human ALS patient specific induced pluripotent stem cells (mutant SOD1 and C9orf72) to oligodendrocyte progenitors and myelinating oligodendrocytes, and compared their gene expression profiles with those from healthy control subjects. We also examined the effects of the mutant ALS gene(s) on axonal myelination in vitro, as well as survival of motor neurons. The role of human oligodendrocytes in ALS pathogenesis will be further discussed.

Disclosures: Y. Li: None. **D. Bergles:** None. **J.D. Rothstein:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.18/P1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CIHR grant MOP 14137

Title: Specific motor-unit changes in perisynaptic schwann cell at nmjs of symptomatic $SOD1^{g37r}$ mice

Authors: *D. ARBOUR[1], E. TREMBLAY[2], E. MARTINEAU[2], R. ROBITAILLE[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of motoneurons and consequent skeletal muscle denervation and neuromuscular junction (NMJ) destruction. A large body of evidence indicates that denervation proceeds in a muscle-specific manner whereby fast functional motor units are preferentially lost compared to slow-twitch muscles. Recent studies have highlighted the implication of glial cells in the disease progression such as activation of axonal Schwann cells at the onset of hind limb paralysis.

However, changes in non-axonal Schwann cells at the NMJ remain ill defined. Perisynaptic Schwann cell (PSCs), glial cells at the NMJ, influence structural stability, integrity and repair of the NMJ and actively decode synaptic activity. Hence, we postulate that PSCs ability to regulate NMJ functions are altered in ALS. NMJs from soleus nerve-muscle preparations of symptomatic (P350 to 380) $SOD1^{G37R}$ mice and their wild type litter mates were used. First, labelling of the three synaptic elements (pre- postsynaptic and PSCs) at symptomatic stage revealed that 25% were denervated, 25% showed signs of poly-innervation and nerve terminal sprouting was often associated with poly-innervation. Second, to test the ability of PSCs to detect synaptic activity, we monitored PSCs calcium responses to endogenous neurotransmitter release. PSCs calcium responses from $SOD1^{G37R}$ mice elicited by nerve stimulation were not different from their littermate, although synaptic efficacy was higher in $SOD1^{G37R}$ mice as revealed by a higher quantal content calculated from intracellular recordings. Ca^{2+} responses induced by ATP were smaller in SOD1 type I fiber type but was larger in type IIa muscle fibers. Furthermore, local application of the mAChR agonist muscarine induced higher calcium responses in PSCs of $SOD1^{G37R}$ mice compared to wild type. More specifically, the amplitude of PSCs calcium responses was larger when associated with fast twitch fiber type IIa than with slow muscle fiber type I in $SOD1^{G37R}$ mice. Since PSCs muscarinic receptors are known to regulate NMJ stability and that fast-twitch motoneurons will degenerate first, it seems that PSCs undergo specific motor unit changes to adapt to the disease progression. Overall, our data suggest that the phenotype of PSCs would not be compatible for NMJ plasticity and repair.

Disclosures: D. Arbour: None. **E. Tremblay:** None. **E. Martineau:** None. **R. Robitaille:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.19/P2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Rings grant #ACT1121 (Conicyt, Chile)

FONDECYT#1110159 (Conicyt, Chile)

Title: Clinical and genetic characterization of a cohort of 30 Chilean patients with dysferlinopathy

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Abstract: Mutations in the dysferlin gene lead to LGMD2B and Miyoshi myopathy among other phenotypes. We describe a cohort of 31 patients, from 25 non-related Chilean families, harbouring point mutations in the DYSF gene. Diagnosis was based on clinical findings or absence of dysferlin in muscle biopsies. Assessment workup consisted of clinical evaluation, Motor Function Measure (MFM) scale, CK level, electrodiagnostic testing, whole body MRI, echocardiogram, spirometry and DYSF gene direct sequencing. Eight mutations were consistently found in the cohort, four of which (c.5979dupA; c.2858dupT; c.2779delG and c.4390G>T) accounted for 82% of the mutations found. In four patients only one mutation was found after complete DYSF gene sequencing. The age at symptom onset ranged from 10 to 33 years (mean 20.8), symptoms manifesting invariably as weakness in the legs, distally (21/31) or proximally (10/31), progressing later to the upper limbs. Mean serum CK level was increased 57(±35) times above normal values. Electrodiagnostic assessment showed normal NCV and repetitive stimulation testing, with distinct degrees and distribution of myopathic changes on needle EMG. Single fibre EMG was normal in six confirmed dysferlinopathy patients. Muscle MRI done in 28/31 patients showed impairment with a similar distribution in all patients despite clinical phenotype. Spirometry showed a mild restrictive defect in 3/18 patients at late stages of disease. Echocardiogram performed in 23/31 patients was within normal range. The clinical spectrum of dysferlinopathy in the series is in agreement with similar cohorts reported. The relative high frequency of some mutations suggests a founder effect for such mutations in the Chilean population. We therefore propose to evaluate the effect these recurrent mutations in vesicle trafficking and cell membrane fusion events, using in vitro cell models such as the RCMH human muscle cell line, muscle primary cultures and myogenic cell lines from patients. We will pay special consideration to prevalent Chilean mutations. Regarding the association of dysferlin with the dihydropyridine receptor and proteins such as annexins and AHNAK that are involved in actin organization, we will also evaluate the role of dysferlin in calcium signals and cortical actin organization.

Disclosures: P.A. Caviedes: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IP protection for RCMH cell line. **C. Castiglioni:** None. **G.A. Di Capua:** None. **L. Woudt:** None. **J. Díaz:** None. **M. Campero:** None. **R. Hughes:** None. **P. González-Hormazábal:** None. **R. Godoy-Herrera:** None. **N. Levy:** None. **M. Krahn:** None. **L. Jara:** None. **J.A. Bevilacqua:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.20/P3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Kirby Foundation

Title: Immunization of mice with muscle specific tyrosine kinase leads to motor nerve alterations

Authors: *V. PATEL[1,2], A. OH[1,2], L. G. SULTATOS[1,2], B. A. WILSON[3], M. HO[3], J. J. MCARDLE[1,2];
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Abstract: Autoantibodies to muscle specific tyrosine kinase (MuSK) and the acetylcholine receptor (AChR) produce distinct forms of myasthenia gravis (MG). In particular, MuSK-MG patients do not exhibit significant loss of AChRs. Furthermore, transmitter release increases and decreases for AChR-MG and MuSK-MG, respectively. These findings suggest motor nerve dysfunction during MuSK-MG. To test this hypothesis, we utilized a model of MuSK-MG produced in mice immunized with rat MuSK ectodomain. C57B6 female mice were injected with 40 μg of MuSK emulsified in 100 μl of equal volumes of PBS and complete Freund's adjuvant once a month for 3 months; controls were injected with vehicle alone. All MuSK injected mice produced antibodies. However, in vivo evaluation revealed variability in the degree of muscle dysfunction. Therefore, to identify severely affected mice we isolated diaphragm-muscle phrenic-nerve preparations and evaluated mechanical responses to nerve stimulation. For 40% of MuSK injected mice twitch tension was significantly reduced from the control value of 4.75 ± 0.19 (n=10) to 2.87 ± 0.20 grams (n=8). In order to study the neuromuscular junction (NMJ) of these mice, Triangularis sterni nerve-muscle preparations were also isolated. Alterations of motor nerve function were noted for 3 groups of NMJs: Group 1 had prolonged endplate currents (EPCs) of reduced quantal content; Group 2 had intermittent failure of EPCs at 20-70 Hz stimulation frequencies; Group 3 had spontaneous but not evoked transmitter release. EPC failures in Group 2 were associated with the absence of nerve terminal currents. Immunohistochemical imaging showed neurofilament-positive swelling as well as abnormal preterminal branching. Both of these alterations would contribute to intermittent as well as complete failure of stimulus-evoked transmitter release. These findings support the hypothesis

that MuSK-MG is associated with motor nerve dysfunction while providing novel insight into the pathophysiology of the disease.

Disclosures: V. Patel: None. **A. Oh:** None. **L.G. Sultatos:** None. **B.A. Wilson:** None. **M. Ho:** None. **J.J. McArdle:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.21/P4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS058510

Title: Investigation of semaphorin3a as a regulator of motor axon reinnervation at the neuromuscular junction

Authors: *J. SHADRACH, B. PIERCHALA;
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Abstract: Motor function is achieved by proper communication between motor neurons and muscle fibers at a specialized synapse called the neuromuscular junction (NMJ). Throughout life the maintenance of the NMJ is an active process that requires the coordinated effort of three main cell types: the presynaptic motor neuron (MN) terminal, the postsynaptic muscle fiber, and terminal Schwann cells (TSCs). Both traumatic injury and neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), can result in acute or chronic NMJ denervation, respectively. Under those conditions denervation triggers a regenerative response in which motor nerve terminals are stimulated to grow and undergo axonal sprouting as they reinnervate muscle fibers. Many studies suggest that reestablishment of neuromuscular connections is mediated by various axonal guidance molecules, of which Semaphorin3A (Sema3A) may be of particular importance. Sema3A is a secreted glycoprotein that binds to a plexin-neuropilin-1 receptor complex and initiates downstream signaling cascades that induce growth cone collapse and axonal repulsion. Although recently it was discovered that TSCs and isolated muscle cells can upregulate Sema3A mRNA in response to nerve and muscle injury, preliminary data we have generated suggests that Sema3A protein is localized to the area surrounding the NMJs under normal, resting conditions and is dramatically decreased in response to both acute, chemically-induced muscle injury and during the later stages of neurodegeneration in the ALS G93A-SOD1 transgenic mouse model. Ongoing work utilizing a newly generated Sema3A-GFP reporter line and other transgenic models are aimed at identifying which cell types within the skeletal muscle

produce Sema3A, what conditions are required for that to occur, and how production of Sema3A functionally impacts reinnervation and/or maintenance of the NMJ.

Disclosures: J. Shadrach: None. **B. Pierchala:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.22/P5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH-R01-NS081118

Title: Responses to VPLo thalamus stimulation in primary motor cortex

Authors: *F. AGNESI[1], A. T. CONNOLLY[1], J. XIAO[1], M. D. JOHNSON[1,2];
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Abstract: The cerebellar-receiving area of motor thalamus has been the primary anatomical target for deep brain stimulation therapy in cases of Essential Tremor. Despite the clinical successes of the therapy, little is known about how motor thalamus stimulation modulates intracortical dynamics within primary motor cortex (M1). In this study, we investigated spontaneous spike activity in M1 during low-frequency (10-30 Hz) and high-frequency (100 Hz) electrical stimulation in the VPLo (ventralis lateralis posterior pars oralis nucleus of thalamus) in a non-human primate. VPLo stimulation was delivered using a tungsten microelectrode inserted into regions responsive to arm movement and susceptible to stimulation-evoked arm movements at low stimulation amplitudes (<30 μA). Behaviorally, low frequency (10-30 Hz) VPLo micro-stimulation never elicited movement, even at higher intensities (60 μA). Higher frequencies (100 Hz) occasionally elicited brief movements at stimulation onset but never induced sustained muscle contractions. Microelectrodes were also inserted into the arm region of M1 (also identified by low threshold stimulation for evoking arm movements), and the activity of single neurons was recorded before, during and after delivery of VPLo micro-stimulation. Peri-stimulus time histograms triggered to each stimulation pulse were constructed and the cumulative sum technique was used to evaluate significance of alteration in firing patterns. We recorded a total of 52 cells and observed two prototypical responses. The first consisted of a sharp increase in the probability of spike activity 3 ms following a stimulus pulse, indicative of stimulating mono-synaptic glutamatergic projections to M1 (10/52 cells). This pattern was most evident for VPLo stimulation at higher frequencies. The second response type consisted of a broad excitation peak

centered at a delay of ~30 ms from the start of the interstimulus interval (10/52 cells), but only at the 10 Hz stimulation frequency. Both responses were observed in the same cell only once. Our data suggests the presence of two pathways excited by VPLo HFS: one direct, inducing short-delayed monosynaptic excitation with low jitter, and one indirect involving multiple synapses.

Disclosures: F. Agnesi: None. **A.T. Connolly:** None. **J. Xiao:** None. **M.D. Johnson:** None.

Poster

530. **Neuromuscular Diseases**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 530.23/P6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Cis-regulatory elements of POLG1 expression with putative tissue-specificity

Authors: *J. P. NIKKANEN, J. PARTANEN, A. WARTIOVAARA;
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Abstract: Polymerase gamma is the only known mitochondrial DNA (mtDNA) replicative polymerase. It consists of a catalytic subunit (POLG1) and two accessory subunits (POLG2). POLG1 mutations are one of the most common causes of mitochondrial disorders and of inherited causes of neurodegeneration, in general. Over hundred disease mutations are found within its coding region. In spite of apparently constitutive expression, the manifestations are highly tissue-specific. One of most common symptom is progressive sensory neuropathy. The basis of the highly specific manifestations is unknown. We have studied POLG1 expression regulation and identified three conserved nervous system specific enhancer modules that are likely to regulate POLG1 tissue-specific expression. We further characterized these enhancers by constructing mouse lines showing the tissue-specific expression pattern throughout development and in adult mice. Two of the enhancers are functional in adult brain and spinal cord, whereas one was found to be specific for the development of the central nervous system. Interestingly, these enhancers locate in the introns of a long intergenic non-coding RNA (lincRNA). We studied the lincRNA and POLG1 transcript levels in different regions of the central nervous system and noticed that they strongly correlate. This was further validated by lincRNA knock-down experiment and therefore we suggest that this lincRNA is regulating POLG1 expression in the central nervous system, possibly together with the characterized enhancer modules. Based on our studies in the spinal cord, we suggest that the subpopulation of neurons of the dorsal horn and dorsal root ganglia, where the enhancers drive expression, might be involved in the

development of sensory neuropathy in POLG1 patients. This is the first study showing tissue-specific expression regulation of an essential mtDNA maintenance protein.

Disclosures: J.P. Nikkanen: None. **J. Partanen:** None. **A. Wartiovaara:** None.**Poster**

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.01/P7

Topic: C.07. Developmental Disorders

Support: Korean NRF Grant 2011-0014258

Korean Health Technology R&D Project, Ministry of health & welfare, A120029

Title: Transient up-regulation of Pax6 increases glutamatergic neuronal differentiation in prefrontal cortex of an animal model of autism

Authors: *K. KIM[1,2], C. CHOI[3], J.-W. KIM[3], M.-R. SONG[5], J. CHEONG[6], C. SHIN[4]; [1]Col. of pharmacy, Col. of Pharmacy, Seoul Natl. Univ., Seoul, Korea, Republic of; [2]SMART Inst. of Advanced Biomed. Sci., Konkuk university, Seoul, Korea, Republic of; [3]SMART Inst. of Advanced Biomed. Sci., [4]Sch. of Med., Konkuk Univ., Seoul, Korea, Republic of; [5]Sch. of Life Sciences, Bioimaging Res. Ctr. and Cell Dynamics Res. Ctr., Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of; [6]Dept. of Pharm., Sahmyook Univ., Seoul, Korea, Republic of

Abstract: Imbalance in excitatory/inhibitory signal in the brain has been suggested as a one of the main pathological features in autism spectrum disorders (ASDs), although the underlying cellular and molecular mechanism is unclear yet. Because excitatory/inhibitory imbalance can be induced by aberration in glutamatergic/GABAergic neuronal subtype differentiation, we investigated the mechanism of dysregulated neuronal differentiation between excitatory and inhibitory neurons in the embryonic and postnatal brain of prenatally valproate-exposed rat offspring, which is often used as an animal model of ASDs. Transcription factor Pax6, implicated in glutamatergic neuronal differentiation, was transiently increased in embryonic cortex by VPA exposure, which resulted in the increased expression of glutamatergic proteins in postnatal brain of offspring. Chromatin immunoprecipitation showed increased acetylated histone binding on *Pax6* promoter region, which may underlie the transcriptional up-regulation of Pax6. Other HDAC inhibitors (HDACi) including TSA and SB but not valpromide, which is devoid of HDACi activity, induced Pax6 up-regulation. Silencing Pax6 expression in cultured rat primary neural progenitor cells demonstrated that up-regulation of Pax6 plays an essential role in VPA-induced glutamatergic differentiation. Blocking glutamatergic transmission with MK-801 or Memantine treatment, and to a lesser extent with MPEP treatment, reversed the impaired

social behaviors and seizure susceptibility of prenatally VPA-exposed offspring. Together, environmental factors may contribute to the imbalance in excitatory/inhibitory neuronal activity in autistic brain by altering expression of transcription factors governing glutamatergic/GABAergic differentiation during fetal neural development, in conjunction with the genetic preload.

Disclosures: K. Kim: None. **C. Choi:** None. **J. Kim:** None. **M. Song:** None. **J. Cheong:** None. **C. Shin:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.02/P8

Topic: C.07. Developmental Disorders

Title: Reduced axonal localization of a Caps2 splice variant impairs axonal release of BDNF and causes autistic-like behavior in mice

Authors: *T. SADAKATA;
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Abstract: Ca2+-dependent activator protein for secretion 2 (CAPS2 or CADPS2) potently promotes the release of brain-derived neurotrophic factor (BDNF). A rare splicing form of CAPS2 with deletion of exon3 (dex3) was identified to be overrepresented in some patients with autism. Here, we generated Caps2-dex3 mice and verified a severe impairment in axonal Caps2-dex3 localization, contributing to a reduction in BDNF release from axons. In addition, circuit connectivity, measured by spine and interneuron density, was diminished globally. The collective effect of reduced axonal BDNF release during development was a striking and selective repertoire of deficits in social- and anxiety-related behaviors. Together, these findings represent the first mouse model of a molecular mechanism linking BDNF-mediated coordination of brain development to autism-related behaviors and patient genotype.

Disclosure.DisclosureBlock:

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.03/P9

Topic: C.07. Developmental Disorders

Support: National Science Council Grant of Taiwan, NSC 100-2320-B-010-033-MY2

Title: Altered neuroplasticity of the amygdala in valproate-induced rat autism model

Authors: *H.-C. LIN[1,2], Y.-H. CHAN[3], P.-S. CHEN[4];
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Abstract: Clinically, exposure of epileptic patients to valproic acid (VPA), an epigenetic regulator, during the period of pregnancy could cause developmental delays and increase the risk of autism in the child. Among the rodent models of autism, prenatal or postnatal exposure to VPA during the critical period had also been demonstrated to lead to autistic phenotypes. Amygdala is the brain area involved in socio-emotional behavior. However, the role of amygdala in autism remains inconclusive. Here, we used 28-35 days valproate (VPA)-induced rat model of autism to observe the autistic phenotypes and evaluate their synaptic characteristics in the lateral nucleus (LA) of the amygdala. The VPA-exposed offspring demonstrated less social interaction and increased anxiety. Slice preparation and electrophysiological recordings of the LA were performed in the VPA- exposed offspring. The magnitude of long term potentiation (LTP) was significantly higher in the VPA-exposed than saline-exposed offsprings. Whole-cell recordings of the LA pyramidal neurons showed an increased miniature excitatory postsynaptic current (EPSC) frequency and amplitude. These results indicated the enhancement of synaptic plasticity and excitatory synaptic transmission in the LA might contribute to autism in the VPA exposed offspring.

Disclosures: H. Lin: None. **Y. Chan:** None. **P. Chen:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.04/P10

Topic: C.07. Developmental Disorders

Support: FP7 EU- NMP4-LA-2009-229289 NanoII

Title: Mechanotransduction of hippocampal neurons: Role of ubiquitin ligase E3a (Ube3a) in neurite contact guidance

Authors: *I. TONAZZINI[1], G. M. VAN WOERDEN[2], S. MEUCCI[1], Y. ELGERSMA[2], F. BELTRAM[1], M. CECCHINI[1];
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Abstract: In the brain, all cells are exposed to extracellular stimuli determined by the micro/nano-environment within which they exist, with the local extracellular domain of each cell orchestrating the wiring of the CNS. In fact, neurite initiation is critically controlled by the establishment/maturation of Focal Adhesions (FAs- protein clusters anchoring integrins to cytoskeleton) that in turn coordinate cell polarity and neurite pathfinding/ motility. Recently groups started investigating the processes that regulate neuronal adhesion and migration, using nano-textured substrates, shown to be capable of tuning neuronal differentiation, polarity and neurite orientation.
Here, we studied the mechanotransduction of hippocampal neurons and the role of ubiquitin ligase E3a (Ube3a), whose loss of function causes the neurodevelopmental disorder Angelman Syndrome (OMIN 105830), in neurite contact guidance during path finding. Recent data suggest that the loss of Ube3a expression is associated with defects in neuronal connectivity and morphology, in several brain areas. To test this further we exploited biocompatible nanogratings (NGs, substrates with patterns of sub-um lines of grooves and ridges) with different topographical characteristics (line-widths ranging from 0.5um to 1um) fabricated by nanoimprint lithography on a cyclic olefin copolymer thermoplastic polymer film. NGs were coupled with Wild-Type (WT) and Ube3a-KO primary mouse hippocampal neurons and neuronal topographical sensing was studied as neurite contact guidance with the aim to compare the capability of WT and Ube3a-KO neurons to read and follow physical directional stimuli. As expected, WT neurons could polarize along the NGs, showing very efficient neurite alignment. Interestingly, in Ube3a-KO neurons mechanotransduction is less efficient, as highlighted by an overall loss of cell polarization and neurite alignment. In order to evaluate if this behavior is due to altered adhesion mechanisms in Ube3a-KO neurons, the activation and maturation of FA complexes were investigated. Because interactions have been found between Focal Adhesion Kinase (FAK) and Paxillin (PAX) and αCaMKII, the protein dysregulated in Ube3a-KO mice**,** we focused our analysis on these proteins.
In summary, we report impaired mechanotransduction in Ube3a-KO neurons, resulting in less efficient neurite contact guidance. Our results are in agreement with the hypothesis that the loss of Ube3a would result in abnormal neuronal connectivity/shaping, leading to pathological

neuronal plasticity. This work was supported by the EU FP7 program, grant no. NMP4-LA-2009-229289 NanoII.

Disclosures: I. Tonazzini: None. **G.M. Van Woerden:** None. **S. Meucci:** None. **Y. Elgersma:** None. **F. Beltram:** None. **M. Cecchini:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.05/P11

Topic: C.07. Developmental Disorders

Support: DoD ARP IDA award AR110134

Title: Gastrointestinal dysfunction mediated by GABAA receptors in the Neuroligin-3R451C mouse model of autism

Authors: *E. HILL-YARDIN[1], M. ELLIS[1], N. OEZGUEN[2], T. SAVIDGE[2], J. C. BORNSTEIN[1];
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Abstract: Gastrointestinal (GI) disorders are common in patients with autism spectrum disorder (ASD) and reduce quality of life. However, despite association of many genes influencing CNS synaptic function, the etiologies of these GI symptoms are unknown. Mutations in the neuroligin family of synaptic adhesion molecules are implicated in ASD disease progression. In order to identify underlying biological mechanisms, information on the expression of ASD candidate genes and the functional impact of the NL3R451C mutation in the enteric nervous system (ENS) is required.
Methods: Human ASD-associated neuroligin mutations were structurally mapped using homology models generated for human NLG1, 2, 3 and 4Y and aligned to core structured regions of the NLGN4-X crystal structure. Protein-protein interfaces were predicted using the InterProSurf web server. Expression of neuroligins and neurexins was assessed in gut and brain cDNA using RT-PCR. Colonic migrating motor complexes (CMMCs) were analysed in isolated colon segments using video imaging techniques and spatiotemporal maps. CMMCs were assessed at baseline and in the presence of GABAA (bicuculline 10 µM and gabazine 10 µM) and GABAB (CGP 54626; 100 nM) receptor antagonists.
Results: The structural domain harbouring R451C contained 5 of 7 identified mutation sites (Arg451 on NLGN3 and Asp429, Asp396, Val403 and Lys378 on Neuroligin 4X), closely juxtaposed to two protein-protein interface patches. We show expression of Nlgn3, related genes

(Nlgn1 and Nlgn2) and neurexin binding partners (Nrxn 1 and Nrxn 2) in the mouse ENS. Further, both bicuculline (n = 16 WT, n = 16 NL3 R451C) and gabazine (n = 11 WT, n = 11 NL3 R451C) reversibly depressed CMMCs in NL3 mice compared to WT while CGP 54626 (n = 8 WT, n = 9 NL3) had no effect. Bicuculline reduced CMMCs in NL3R451C compared to WT colon (median difference: 6 contractions, 95% CI: 1, 12; p = 0.03). Gabazine also reduced CMMC frequency in NL3R451C mice compared to WT (median difference: 5 CMMCs, 95% CI: 2, 9; p = 0.009). In control conditions, CMMC frequency was identical in WT (n = 70) and NL3R451C (n = 69) tissues; in both WT and NL3 R451C the median number of CMMCs was 4 (WT: 95% CI: 1, 11; NL3 R451C: 95% CI: 1, 15; median difference 1, 95% CI -2, 4; p = 0.509). Conclusion: Positional association of R451C with other clinically identified mutations suggest that a functionally conserved domain in the neuroligin family may be targeted in ASD. We show that the R451C knock-in mutation in the NL3R451C mouse model of ASD causes colonic motility dysfunction via a GABAA receptor mediated mechanism. These data implicate altered enteric synaptic function as a primary underlying cause of GI disorders in ASD.

Disclosures: E. Hill-Yardin: None. **M. Ellis:** None. **N. Oezguen:** None. **T. Savidge:** None. **J.C. Bornstein:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.06/P12

Topic: C.07. Developmental Disorders

Support: Simons Foundation Grant 249475

Title: The Role of MAPK/ERK signaling in ASD pathogenesis associated with copy number variation in 16p11.2 deletion mice

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Abstract: Copy number variations (CNVs) of human chromosome 16p11.2 are linked to autism spectrum disorders (ASD), where deletions or duplications of this region account for 1% of all ASDs. The extracellular signal-regulated kinases, ERK1 and ERK2, are the central elements of one of the most prominent signaling pathway governing neural development and synaptic plasticity and are genetically linked to ASDs and cognition. The 16p11.2 human locus contains 27 genes, including the ERK1 gene (MAPK3) and Major Vault Protein (MVP) gene. MVP regulates signaling through the ERKs. A mouse model of the human 16p11.2 microdeletion (the

chr7qF3-deficient mouse) recapitulates some of the behavioral pathology associated with ASD patients. However, the pathophysiological and biochemical mechanisms underlying these abnormalities are unexplored. We propose that altered expression of one or more genes in the 16p11.2 region converges onto the ERK/MAP kinase signaling pathway resulting in deficits associated with ASD.
We developed constitutive ERK1 knockout (ERK1 KO) and conditional ERK2 knockout (CKO) mouse models. These models exhibit deficits which partially phenocopy the behavioral deficits observed in ASD patients. We previously established a mechanism by which ERKs regulate corticogenesis, where loss of ERK activity leads to defects in progenitor proliferation resulting in altered cortical cytoarchitecture, abnormal physiology and behavior. Given the convergence of genetic evidence onto the ERK pathway and their role in neurogenesis we examined the 16p11.2del ASD mice for abnormalities we previously linked to neurodevelopmental disorders of the MAP kinase pathway, including Autism.
We report that: ERKs are hyperactivated in 16p11.2del ASD mice, resulting in aberrant neurogenesis and deficits in postnatal brain cytoarchitecture. Specifically, we observe a 30% decrease in upper cortical projection neurons (Brn1+, SatB2+ neurons) and a 10% increase in layer VI cortico-thalamic, Tbr1+ neurons. Additionally, during mid-neurogenesis, we detect a significant increase in progenitor proliferation in the ventricular and subventricular zone as evidenced by elevated PH3 staining. However, the total number of Tbr2+ intermediate progenitors as well as their generation from Pax6+ radial glia is significantly decreased. We examined the ASD mice for changes in the cell cycle regulator p27KIP1, which is directly targeted by the ERKs and mediates cell cycle progression. We observed a significant decrease in p27Kip1 by Western analysis as well as immunohistochemistry. Furthermore, we report that the 16p11.2del ASD mice are hyperactive and exhibit anxiety-like behaviors.

Disclosures: J. Pucilowska: None. **J. Vithayathil:** None. **G.E. Landreth:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.07/P13

Topic: C.07. Developmental Disorders

Title: Reduced GABAergic interneurons density and functional alterations in the visual cortex of Engrailed-2 knockout mice, a murine model for autism

Authors: *M. ALLEGRA[1,2], S. GENOVESI[3], P. SGADÒ[3], M. CENNI[1], Y. BOZZI[3], M. CALEO[1];

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Abstract: The maturation of the GABAergic system is a crucial determinant of cortical development during early postnatal life, when sensory circuits undergo a process of activity-dependent refinement. An altered excitatory/inhibitory balance has been proposed as a possible pathogenic mechanism of various neurodevelopmental disorders, such as Autism Spectrum Disorders (ASD) and epilepsy.
The homeobox-containing transcription factor Engrailed-2 (En2) has been implicated in the pathogenesis of ASD. Mice lacking En2 (En2-/- mice) display anatomical and behavioural "autistic-like" features, as well as increased seizure susceptibility, a clinical feature frequently observed in ASD patients.
Here we have explored the relationship between GABAergic innervation and maturation of cortical function in En2-/- mice. To address this issue, we have exploited the good knowledge of the anatomical and functional features of the mouse visual system.
First, we analyzed the profile of GABAergic interneurons in the visual cortex. En2-/- mice, when compared to age-matched wild-type (WT) controls, showed a reduction in the number of parvalbumin (PV), somatostatin (SST) and neuropeptide Y (NPY) positive neurons. Quantitative real time RT-PCR experiments confirmed the decreased expression of PV, SST and NPY in En2-/- mice. We next investigated whether the reduced number of GABAergic interneurons results in an altered maturation of visual system in En2-/- mice. At the anatomical level, En2-/- mice displayed a normal eye-specific segregation the retino-geniculate pathway. Visual Evoked Potentials (VEPs) and single units recordings were performed to assess the functional properties of primary visual cortex (visual acuity, contrast threshold and binocularity) in adult WT and En2-/- mice. While basic physiological parameters (acuity, contrast, response latency) developed normally, we found a significantly increased binocularity in En2-/- mice, pointing to a physiologically more immature state of the visual cortex.
These data suggest that a deficient GABAergic inhibition in the visual cortex of En2-/- mice is involved in the altered maturation of cortical binocularity. This study strengthens the notion that GABAergic system dysfunction contributes to cortical circuit alterations in murine models of ASD.

Disclosures: M. Allegra: None. **S. Genovesi:** None. **P. Sgadò:** None. **M. Cenni:** None. **Y. Bozzi:** None. **M. Caleo:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.08/P14

Topic: C.07. Developmental Disorders

Support: NSC 99-2321-B-006 -002

Title: Increased cell proliferation and reduced neural differentiation in the developing hippocampus following postnatal exposure to sodium valproate

Authors: C.-W. CHENG[1], W.-H. WANG[1], C.-S. YANG[3], *S.-F. TZENG[2];
[1]Life Sci., [2]Natl. Cheng Kung Univ., Tainan City, Taiwan; [3]Ctr. for Nanomedicine Res., Natl. Hlth. Res. Institutes,, Zhunan, Taiwan

Abstract: Autism is a neurodevelopmental disorder with impairments in social interaction, repetitive behaviors and interests, as well as cognitive delays. However, the molecular mechanism remains largely unknown. A neurobehavioral model of autism in rodents receiving prenatal or postnatal exposure of sodium valproate (VPA) has been characterized. In our laboratory, we used the model of male rat pups receiving 200 mg/kg of VPA via a bolus of intraperitoneal injection at postnatal day 7. The results from elevated the plus-maze test at 2, 4 and 6 weeks post injection showed the VPA-injected rats exhibited impaired social interaction and anxiety. Through the open field test, we found that VPA-treated rats displayed an anxious behavior and abnormal locomotion. Given the fact that cortex and hippocampus are associated with autistic behaviors, we examined cellular and molecular alterations in the hippocampus of rats at 24 h, 2 w, 4 w, and 6 w. An increased level of acetylated histone H3 was detected at 24 h in cortex and hippocampus of rat pups receiving VPA injection when compared to that observed in vehicle-treated rats. $BrdU^+$-cells or $Ki67^+$-cells in the hippocampus of rats were also increased at the four observed time points after VPA exposure. In addition, neuronal differentiation gene, NeuroD, was decreased in the hippocampus of VPA-treated rats at the four detected time points. Olig1 and olig2 gene expression was also downregulated, but GFAP mRNA expression was increased in VPA-treated group. Collectively, autistic phenotype induced by postnatal VPA exposure caused increased cell proliferation and reduced neural differentiation in rat hippocampus, which could contribute to social deficit and anxiety.

Disclosures: C. Cheng: None. **W. Wang:** None. **S. Tzeng:** None. **C. Yang:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.09/P15

Topic: C.07. Developmental Disorders

Support: NIMH R01 MH39805

Boyd and Elsie Welin Professorship

Title: Serum antibody changed in MAOA deficiency autism spectrum disorder mice

Authors: *K. CHEN[1], J. C. SHIH[1,2], C.-S. CHEN[3], F. SUTANDY[3];
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Abstract: Monoamine oxidase A (MAOA) is an enzyme that metabolizes xenobiotic monoamines including serotonin, dopamine and norepinephrine. The MAOA deficient human and knock-out mouse has impairment in social interactions, and autism spectrum disorder phenotype (1, 2). MAOA is highly expressed in digestive system epithelial cells. We suspect the MAOA KO mice may also have abnormal immune response. Here we performed a comprehensive study by using human proteome microarray containing ~17,000 non-redundant human proteins to compare the serum profiles of wild type and MAOA KO mice. Sixteen serum samples from two different groups were collected and probed into the human proteome chip. Twenty six proteins were identified as up or down regulated proteins in the mutant which were most likely corresponded to MAOA related pathways. We further profiled the identified proteins to screen out any protein which might be related to the development of autism. Among them, we were interested on a protein from retinoic acid pathway cellular retinoic acid binding protein 2 (CRABP2). This protein was previously reported for its implication in many neural diseases as its important function in neuron differentiation. Moreover neural differentiation is a critical process which is altered in most autism cases. Based on our current findings we hypothesized that this protein and retinoic acid pathways were the missing gap between MAOA and autism development. Further experiment will be conducted in the near future to clearly demonstrate the role of CRABP2 in MAOA related autism development.
The discovery of changed serum antibody profile in MAOA KO ASD model offered possible application of serum antibody as a diagnostic method for ASD and other neuropsychological diseases.
Reference
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2.
De nove microdeletion of Xp11.3 exclusively encompassing the MAOA and B genes in a male with episodic hypotonia. (2012) O'Leary et al. Eur J Med Genet 55:349.

Disclosures: K. Chen: None. **J.C. Shih:** None. **C. Chen:** None. **F. Sutandy:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.10/P16

Topic: C.07. Developmental Disorders

Support: Foundation for Polish Science Homing Plus Grant

Title: Mice lacking MMP-9 show stereotypic behaviors, but no social impairment

Authors: *K. Z. MEYZA, A. PUSCIAN, T. LEBITKO, E. KNAPSKA;
Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Matrix metalloproteinase-9 (MMP-9), known to be involved in regulation of synaptic plasticity, has recently been implicated to play a role in development of autism spectrum disorder (ASD). Increase in its expression in the amniotic fluids has been correlated with the consequent diagnosis with ASD in a population of Danish children (Abdallah et al. 2012) as well as with behavioral impairments in Fragile X syndrome patients. Since the amount of expressed MMP-9 and its activity are crucial for control of learning (and unlearning), which seems to be altered in ASD patients, we suggested that the lack of this enzyme should cause autism-like behaviors in MMP9KO mice. To test that, we used the Intellicage system allowing for observation of place preference learning and reversal without much human intervention. Already at the adaptation level we found that females lacking MMP-9 showed stereotypic bias towards a chosen cage corner, usually observed in mouse models of autism. The MMP9KO males behavior however, was similar to that of WT mice. To test their social behavior we tested the animals in social approach paradigm (3-chambered apparatus) to find that KO mice did not differ from WT in either time spent on the social side of the apparatus, nor in the duration of sniffing an unfamiliar animal during the test session of this paradigm. The lack of MMP-9 seems to affect the stereotypic behaviors and learning abilities rather than social preference.

Disclosures: K.Z. Meyza: None. **A. Puscian:** None. **T. Lebitko:** None. **E. Knapska:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.11/P17

Topic: C.07. Developmental Disorders

Support: PRIN 2008 grant # 200894SYW2_002

Marie Curie COFUND - progetto Trentino - Encort

Title: Transcriptome profiling in Engrailed2 knockout mice reveals convergent molecular pathology associated with ASD

Authors: *P. SGADÒ[1], G. PROVENZANO[1], V. ADAMI[1], E. DASSI[1], G. ZUNINO[1], S. GENOVESI[1], S. CASAROSA[1], Y. BOZZI[1,2];
[1]Ctr. For Integrative Biol. - Univ. of Trento, Trento, Italy; [2]CNR Neurosci. Insitute, Pisa, Italy

Abstract: Genome-wide association studies indicated the human Engrailed2 gene (EN2) as a candidate gene for autism spectrum disorders (ASD). Recent studies showed that En2 knockout mice (En2-/-) represent a suitable animal model to study the neurodevelopmental basis of ASD. En2-/- mice display cerebellar hypoplasia and a reduced number of Purkinje cells, as well as "ASD-related" behaviors, such as decreased attitude to play, spatial learning deficits and increased seizure susceptibility. En2 controls the patterning and neuronal differentiation in the midbrain/hindbrain region, where it is mainly expressed. However, our recent data indicate that En2 is also expressed in the adult hippocampus and cerebral cortex, suggesting that it might also control the function of telencephalic regions. Using qPCR and immunohistochemistry for GABAergic markers, we found that En2-/- mice have a partial loss (about 30%) of GABAergic interneurons in the hippocampus and somatosensory cortex, as compared to WT mice. These anatomical changes are accompanied by a profound alteration of the gene expression profile in the En2-/- forebrain. We compared the cerebellar expression profile with that of the hippocampus using microarray experiments. Based on our statistical criteria we found 842 differentially expressed genes in the cerebellum of En2-/- mice compared to their control littermates. Among these 403 and 439 showed up- and down-regulation, respectively. In the hippocampus we found 862 differentially expressed genes, among those 378 were up-regulated and 484 were down-regulated in En2-/- mice. To validate differential expression we selected 20 genes for qPCR analysis and demonstrate that the expression differences reported by qPCR for the tested genes correlated with the microarray data. To assess the functional consequences of En2 ablation we analyzed enrichment of the differentially expressed genes based on the mouse phenotype database and found for the hippocampus a significant enrichment for "seizure-related" phenotypes. Remarkably, when analyzed specifically for association with autism-related genes of the SFARI repository (sfari.org), the differentially expressed genes showed significant overrepresentation of known autism susceptibility genes both in the cerebellum and in the hippocampus. In details, 18 and 25 of the differentially expressed genes, respectively in cerebellum and in the hippocampus, were present in the SFARI ASD genes.
The molecular evidence we collected suggest that En2 mutation cause alterations in several

pathways associated to ASD, suggesting a prominent role of En2 in the dysregulation of convergent biological pathways in ASD.

Disclosures: P. Sgadò: None. **G. Provenzano:** None. **V. Adami:** None. **E. Dassi:** None. **G. Zunino:** None. **S. Genovesi:** None. **S. Casarosa:** None. **Y. Bozzi:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.12/P18

Topic: C.07. Developmental Disorders

Support: NIH Grant 5R01HD069560 to CMP

NIH Grant R01HD69560-0251 to CMP/TCJ

Autism Speaks Translational Postdoctoral Fellowship to HES

Autism Speaks Pilot Award to CMP

NIH R21HD065290 to CMP

Title: Altered synaptic plasticity and abnormal behaviors in Shank3 exon 4-9 mutant mouse model of autism

Authors: *T. C. JARAMILLO, H. E. SPEED, J. REIMERS, Z. XUAN, S. LIU, C. M. POWELL;
Neurol. & Neurotherapeutics, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Shank3 (aka Pro-SAP2) is a multi-domain, synaptic scaffolding protein that organizes glutamate receptors and scaffolding proteins in the postsynaptic density of excitatory synapses. Clinical studies suggest that ~0.5 % of autism spectrum disorder (ASD) cases may involve Shank3 mutations/deletion, suggesting a role for this gene in autism pathogenesis. Patients with Shank3 mutations exhibit deficits in cognition along with delayed speech and repetitive and obsessive/compulsive-like (OCD-like) behaviors. To examine how mutation/deletion of Shank3 might alter brain function leading to ASD, we have created mice with deletion of exons 4-9, corresponding to the ankryin repeat domain, a region implicated in ASD patients with mutations or translocation breakpoints.
Consistent with previous reports, we find that homozygous deletion of exons 4-9 (Shank3e4-9) results in a loss of only the highest molecular weight isoform of Shank3 (Shank3α).

Behaviorally, Shank3e4-9 KO mice displayed increased repetitive grooming, deficits in object recognition learning and memory, abnormal ultrasonic vocalizations, normal coordination on the rotarod, and normal sociability. Biochemical analysis of synaptoneurosome fractions revealed striatum-specific deficits in GluN2B and GluR2/3 subunit expression that coincided with a reduction in NMDAR/AMPAR ratio in excitatory inputs onto striatal medium spiny neurons. There were also reduced levels of Homer1b/c and PSD-95. Furthermore, Shank3e4-9 KO mice displayed reduced hippocampal LTP, enhanced DHPG LTD and normal baseline synaptic transmission. Thus, complete loss of the highest molecular weight isoform of Shank3 in mice is sufficient to induce behavioral deficits loosely analogous to those seen in many patients with Phelan-McDermid Syndrome or ASD. Additionally, biochemical and physiological changes in Shank3-E4-9 mice suggest region-specific roles for the Shank3α isoform in regulating AMPAR and NMDAR subunit localization or expression and function. Our data suggest that replication of phenotypes across laboratories in similar genetic models will ultimately reveal the most robust behavioral phenotypes, the most relevant functional abnormalities, and ultimately the most promising therapeutic targets.

Disclosures: T.C. Jaramillo: None. **H.E. Speed:** None. **J. Reimers:** None. **Z. Xuan:** None. **S. Liu:** None. **C.M. Powell:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.13/Q1

Topic: C.07. Developmental Disorders

Title: Changes in molecular motors in doublecortin deficient mice

Authors: *X. FU, K. J. BROWN, J. K. JAISWAL, J. S. LIU;
Neurosci., Children's Natl. Med. Ctr., Washington, DC

Abstract: Mutations of the human doublecortin (DCX) gene cause X-linked lissencephaly and double cortex syndrome. The molecular mechanism of DCX effects is not fully understood. As a microtubule-associated protein, Dcx has a novel function in the regulation of molecular motors. Specifically, Dcx is essential for the function of Kif1a, a kinesin-3 motor protein that traffics synaptic vesicles. Because of the severity of the Dcx/Dclk1 phenotype (defects in neuronal migration, axon and dendrite formation), we hypothesized Dcx regulation of motors in addition to Kif1a. We performed a semi-quantitative proteomic analysis of the corpus callosum in mice mutant for Dcx. In axons from mice mutant for Dcx, differences are found in several motor

proteins including dynein, a major motor protein transporting cargo along microtubules towards the minus ends. Further analysis using immunostaining, live imaging, immunoprecipitation, and Western Blotting indicate that Dcx inhibits dynein mediated retrograde transport. Therefore, although Dcx enhances forward trafficking by kinesins, Dcx inhibits retrograde dynein transport. This finding further demonstrates the critical roles of Dcx in motor trafficking and demonstrates why disruption of Dcx leads to abnormal neuronal development.

Disclosures: X. Fu: None. **J.S. Liu:** None. **K.J. Brown:** None. **J.K. Jaiswal:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.14/Q2

Topic: C.07. Developmental Disorders

Support: NIH 1R01 NS063009

Title: Cerebellar pathology results in compensatory neural adaptations within cerebellar-prefrontal cortex pathways involved in modulating cortical dopamine release: Relevance to Autism-related behavioral disorders

Authors: E. MCKIMM[1], B. CORKILL[1], D. HECK[2], D. GOLDOWITZ[3], G. MITTLEMAN[1], *C. D. BLAHA[1];
[1]Dept. of Psychology, Univ. of Memphis, MEMPHIS, TN; [2]Dept. of Anat. & Neurobio., Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN; [3]Child and Family Res. Inst., Ctr. for Mol. Med. and Therapeutics, Dept. Med. Genet., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Autism is characterized by at least two neural abnormalities: cerebellar pathology that includes Purkinje cell loss or functional dysregulation as well as compensatory changes in prefrontal cortex (PFC) dopamine (DA) transmission. The inter-relationship between these two abnormalities is unknown. We have previously shown that PFC DA release evoked by electrical stimulation of the cerebellar dentate nucleus (DN) is attenuated in Lurcher (Lc) mutant mice lacking Purkinje cells (Mittleman et al. 2008 Synapse). A similar loss of PFC regulation was also observed in FMR1 mutant mice showing cerebellar neuropathology involving Purkinje cells. In both mutant mice the PFC dysregulation was directly related to disruption in glutamate (GLU) neurotransmission in DN pathways projecting to the PFC via (**1**) the VTA or (**2**) mediodorsal (Thmd) and ventrolateral (Thvl) thalamic nuclei (Rogers et al. 2011 Synapse). Finally we have also shown that there is a decrease in the amount of localized GLU release in these two circuits (McKimm et al. SfN 698.01). Here we investigated the hypothesis that the disruption in GLU

release is accompanied by postsynaptic developmental adaptation in the two circuits mediating PFC dopamine release. We used fixed potential amperometry in combination with carbon fiber electrodes in urethane anesthetized (1.5g/kg i.p.) wildtype and Lc mice. We stimulated the Thmd, Thvl, or the VTA (50 pulses; 50 Hz) and recorded PFC DA release. Results indicated that there was a greater increase in DA release observed in the PFC while stimulating the VTA in Lc mutant compared to wildtype mice (0.087:0.027nA; [$F(1,5) = 15.6$, $p = .01$]. Conversely, no significant differences were seen with Thmd or Thvl stimulation-evoked PFC DA release between the Lc and wildtype mice {[$F(1,4) = 6.85$, $p = .06$]; [$F(1,5) = .03$, $p = .87$]}. These findings suggest that developmental loss of cerebellar Purkinje cells results in a postsynaptic developmental adaptation in the DN-VTA-PFC circuit. This shows that, although local GLU release is reduced in the VTA, DA release in the PFC is enhanced. This result is consistent with a compensatory enhancement of dopaminergic neurotransmission in the VTA to PFC pathway. In the thalamic pathway, despite a reduction in DN-Th-PFC Glu transmission, no observed compensation occurs postsynaptically in the Thmd or Thvl. Considered together, these compensatory changes, or lack thereof, that result in a change in the relative balance between these two circuits modulating PFC DA release may have implications in understanding the cognitive deficits as well as impairments in social reward commonly observed in autism.

Disclosures: E. McKimm: None. **C.D. Blaha:** None. **D. Heck:** None. **B. Corkill:** None. **D. Goldowitz:** None. **G. Mittleman:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.15/Q3

Topic: C.07. Developmental Disorders

Support: MIUR PRIN n.2006058195

MIUR PRIN n.2008BACT54_002

It. Min. Health n.RFPS-2007-5-640174

Autism Research Institute

Autism Speaks

IMIJU EU-AIMS n.115300

Title: Elevated urinary p-cresol in autism spectrum disorder: Human, rodent and cellular studies

Authors: *S. GABRIELE[1,2], R. SACCO[1,2], S. CERULLO[1], C. NERI[2,3], A. URBANI[2,3], T. PASCUCCI[2,4], C. BRAVACCIO[5], M. RICCIO[6], L. DE MAGISTRIS[7], C. BARTHELEMY[8], F. BONNET-BRIHAULT[8], G. TRIPI[8,9], R. PIACENTINI[10], C. GRASSI[10], A. M. PERSICO[1,2,11]; [1]Univ. Campus Biomedico, Rome, Italy; [2]IRCCS Fondazione Santa Lucia, Rome, Italy; [3]Intrnl. Med., Univ. "Tor Vergata", Rome, Italy; [4]Dept of Psychology and Ctr. "Daniel Bovet", Univ. "Sapienza", Rome, Italy; [5]Dept of Translational Med. Sci., Univ. "Federico II", Naples, Italy; [6]Dept of Physical and Mental Hlth. and Preventive Med., [7]Dept Magrassi-Lanzara, Gastroenterology, Second Univ. of Naples, Naples, Italy; [8]Imagerie et cerveau INSERM : U930, Univ. François Rabelais, Hôpital Bretonneau, Tours, France; [9]Dept PROSAMI, Univ. of Palermo, Palermo, Italy; [10]Inst. of Human Physiol., Catholic Univ. S.H., Rome, Italy; [11]Mafalda Luce Ctr. for Pervasive Developmental Disorders, Milan, Italy

Abstract: P-cresol (4-methylphenol) does not derive from human metabolism. It can be absorbed through the skin, GI or respiratory tracts, but its main source consists in gut bacteria (especially some clostridia) able to push the fermentation of tyrosine up to p-cresol. We found elevated urinary amounts of p-cresol in autistic children up to 8 years of age (Altieri et al., Biomarkers 16:252-260, 2011). These initial findings were replicated and extended by measuring total urinary p-cresol and its derivatives p-cresylsulphate and p-cresylglucuronate using HPLC-fluorimetric detection in 33 French autistic children and 33 matched controls. Urinary p-cresol and its derivatives were all significantly elevated in French ASD cases compared to controls ($p<0.05$). This difference was significant in 18 case-control pairs younger than age 8 ($P<0.05$), but not in 15 pairs of older children ($P=0.211$), although two 8-yo children displayed very high amounts. No correlation was found with clinical severity. In another sample including 32 Italian ASD children and 16 controls, we assessed in parallel intestinal permeability using the LA/MA test, presence of Clostridial species and toxinA in the feces, stool habits and recent antibiotic use. No case of gut infection with C. difficile was detected and no correlation was found with intestinal permeability, but a trend with intestinal transit time was detected. Acute behavioral effects were assessed in rodents administering p-cresol 1 mg/Kg (N=4), 10 mg/Kg (N=4), or vehicle (N=4) i.v. to 12 male BTBR mice at P60, using open field, elevated plus maze and object recognition test. Acute p-cresol largely increases time spent in the open arms of the plus maze and decreases exploration of novel objects at the object recognition test (both $P<0.05$), with no effect in the open field. Finally, calcium-imaging experiments using Fluo-4-AM in cultured cortical neurons demonstrate that p-cresol (40.7 mg/L) does not affect resting intracellular calcium concentration, while significantly antagonizing caffeine (20mM)-induced calcium release through ryanodine receptors (-67%; $P<0.001$). Urinary p-cresol is thus elevated in autistic children prior to and including age 8, possibly reflecting slow intestinal transit time and acutely modulating behaviour.

Disclosures: S. Gabriele: None. **R. Sacco:** None. **S. Cerullo:** None. **C. Neri:** None. **A. Urbani:** None. **T. Pascucci:** None. **C. Bravaccio:** None. **M. Riccio:** None. **L. De Magistris:** None. **C.**

Barthelemy: None. **F. Bonnet-Brihault:** None. **G. Tripi:** None. **R. Piacentini:** None. **C. Grassi:** None. **A.M. Persico:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.16/Q4

Topic: C.07. Developmental Disorders

Support: NIH 1P50MH096891-01

Title: *In vitro* voltage sensitive dye imaging study of a role of NMDA on amygdalar-striatal functional connectivity

Authors: *D. BOHORQUEZ, R. WHITE, K. ZHU, G. CARLSON;
Univ. of Pennsylvanina Perelman Sch. of Med., Philadelphia, PA

Abstract: The amygdala is made up of a number of nuclei that interconnect to many areas of the brain to mediate learning, social interactions, and anxiety. While the anatomical connectivity has been well studied the functional connectivity is not well understood. Strong gamma-band coherence has been found during certain learning tasks between the basal-lateral amygdala (BLA) and striatum. In order to assay functional connectivity between striatal areas medial to the amygdala and the BLA we made coronal slices through the adult mouse brain that captured these areas. These slices were died with voltage sensitive dye and imaged a 1kHz. Following stimulation of the BLA, after the LA, the strongest activity appeared to be the striatal areas directly medial to the BLA. By contrast the central medial amygdala rarely generated robust responses to be either BLA or LA stimulation. This strong striatal response was delayed by 2 to 3 msec consistent with monosynaptic response to the BLA stimulation. To test the ability of this synaptic connection to propagate high frequency activity into the striatum gamma-band bursts were generated in the BLA. These generated both sustained and high frequency ensemble activity in the striatum. In order to explore the role of NMDA receptors in these components AP5 and D-cyclo-serine was applied. Surprisingly, AP5 had very little effect on the response, yet D-cyclo-serine appears to reduce activity. These data suggest a NMDA receptor dependent role on inhibitory components modulating amygdalar activity.

Disclosures: D. Bohorquez: None. **K. Zhu:** None. **R. White:** None. **G. Carlson:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.17/Q5

Topic: C.07. Developmental Disorders

Title: Social behaviors can be assessed with computer vision in mouse models of disease in a home cage environment

Authors: *T. HANANIA, P. KABITZKE, M. MAZELLA, I. FILIPOV, V. ALEXANDROV, D. BRUNNER;
PsychoGenics Inc., TARRYTOWN, NY

Abstract: The assessment of social behavior has become critical for the phenotyping of animals models of autism and schizophrenia. Scoring of videos is both time consuming and subjective, causing inter-observer and between-studies variability, thus emphasizing the need for automated scoring.
Computer vision classification of social behavior is, on the other hand, challenging, due to the frequency of occlusions and contacts between mice. We have solved the problem of occlusion with sophisticated computer vision techniques and individual tags. Careful choice of algorithms also allows us to analyze videos in almost real-time, by minimizing CPU time. Our choice of lighting and camera sensitivity also allow us to track mice during the dark circle and to score their behavior 30 times a second, 24 hours a day, up to 6 days continuously. Assessment of social behavior in a non-stressful environment can also produce qualitatively different phenotypes, as the behavior is not confounded with anxiety or a response to manipulation. We will present data obtained with mouse models of autism, that demonstrate the utility of the system.

Disclosures: T. Hanania: A. Employment/Salary (full or part-time):; PsychoGenics Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Partnership with ROCHE. **P. Kabitzke:** A. Employment/Salary (full or part-time):; PSYCHOGENICS. **M. Mazella:** A. Employment/Salary (full or part-time):; PSYCHOGENICS. **I. Filipov:** A. Employment/Salary (full or part-time):; psychogenics. **V. Alexandrov:** A. Employment/Salary (full or part-time):; Psychogenics. **D. Brunner:** A. Employment/Salary (full or part-time):; Psychogenics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Psychogenics, AMylin.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.18/Q6

Topic: C.07. Developmental Disorders

Title: Emotional pertubations in an environmentally induced animal model of autism

Authors: *A. BANERJEE, J. A. LUONG, S. K. LELLA, B. L. SAULS, C. ENGINEER, M. P. KILGARD, J. E. PLOSKI;
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Abstract: Autism Spectrum Disorders (ASD) are complex neurodevelopmental disorders characterized by core symptoms including repetitive behavior, impaired social interactions and deficits in social communication. Apart from these core symptoms, a significant number of ASD individuals display maladaptive emotional responses. For example numerous studies indicate that ASD individuals display higher levels of anxiety and some studies indicate that ASD individuals are impaired in their ability to be fear conditioned. Therefore we sought to further examine innate fear and emotional learning utilizing an environmentally induced animal model of ASD. This model focuses on progeny from pregnant rats exposed to the known teratogen, valproic acid (VPA) on day 12.5 of gestation. Specifically we exposed dams to either one of two different doses of VPA (500 and 600 mg/kg) or vehicle on day 12.5 of gestation. Resultant progeny at 60 days of age were examined for innate fear and changes in locomotion using an open field test. We then auditory fear conditioned these rats to a 5 kHz 75 dB tone. Our preliminary data indicates that embryonic exposure to VPA, at both doses enhances anxiety as measured as decreased center entries in the open field test, with no changes in overall locomotion. Animals exposed to 500 mg/kg VPA displayed normal acquisition of fear conditioning, but exhibited reduced extinction of fear memory - data consistent with previously published reports. However we observed that rats exposed to 600 mg/kg of VPA exhibited a significant reduction in acquisition of fear conditioning. To determine if the decrease in fear conditioning was due to a sensory deficit, we examined pain perception and hearing in VPA exposed rats. VPA exposed rats exhibited normal pain sensitivity in a hot plate test and exhibited normal hearing to a 5 kHz, 75 dB tone as determined by normal spike firing within the auditory cortex during exposure to 5kHz tones presented at varying degrees of loudness up to 75 dB. Collectively these data indicated that the reduced acquisition of fear learning is not likely due to sensory deficits, but rather due to deficits in learning. To examine the molecular basis of VPA induced impairment in fear learning in animals exposed to VPA (600 mg/kg), we performed whole genome gene expression analysis using DNA microarrays on amygdala RNA from rats exposed to VPA and vehicle. The microarray data indicated that VPA exposed rats may have dysfunctional glutamatergic signaling within the amygdala. We are currently investigating the

hypothesis that deficits in glutamatergic signaling underlie at least in part the impairment in emotional learning in animals exposed to VPA.

Disclosures: A. Banerjee: None. **J.A. Luong:** None. **S.K. Lella:** None. **B.L. Sauls:** None. **C. Engineer:** None. **M.P. Kilgard:** None. **J.E. Ploski:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.19/Q7

Topic: C.07. Developmental Disorders

Support: NIH R15 DA029544

Charles and Johanna Busch Biomedical Research Award and The NJ Commission on Spinal Cord Research CSCR11IRG011

Title: Altered learning and novelty-seeking behavior in adult mice lacking the neuropilin 2 gene

Authors: *M. W. SHIFLETT[1], M. GAVIN[2], T. S. TRAN[2];
[1]Psychology, Rutgers Univ. Newark, NEWARK, NJ; [2]Dept. of Biol. Sci., Rutgers Univ., Newark, NJ

Abstract: The proper formation of neural connectivity depends critically upon the spatially and temporally coordinated regulation of neuronal morphology, and is essential for the precise and complex functions of neural networks controlling appropriate behavior in the animal. However, a large gap in knowledge exists between the molecular mechanisms controlling neuronal morphogenesis and synapse formation to the change in behavior output. Class 3 secreted semaphorins and their neuropilin and plexin receptors are implicated in a large number of neural developmental events, including axon guidance, dendritic branching, excitatory synapse formation, and target selection. Previously, we showed that the secreted semaphorin Sema3F and its Npn2/PlexA3 receptor complex are required for normal hippocampal and layer 5 cortical neuron dendritic spine morphology and excitatory synapse transmission. Here, we investigate the effects on cognition and behavior of altered semaphorin signaling, by testing mice deficient in the gene encoding Npn2. We found that Npn2 knockout mice exhibit altered behavior on novelty-seeking tasks. Specifically, Npn2-/- mice showed no preference for novel social and non-social stimuli as compared with wild type age-matched littermate controls. Furthermore, Npn2 knockout mice were impaired in an operant discrimination learning task, suggesting a fundamental impairment in memory formation. Additionally, Npn2 null animals exhibited

increased anxiety-related behaviors in the open field and tail-suspension tests. We found no effects of the knockout on locomotor behavior or olfactory habituation/dishabituation, indicating intact sensorimotor processing in Npn2-/- animals. We are currently expanding our behavioral tests to analyze Sema3F and PlexA3 knockout mice. Thus, our results support novel roles for secreted semaphorin signaling in controlling complex behaviors in adult mice and underscore the necessity of understanding the mechanisms of how changes in neuronal morphology and connection may lead to altered complex behavior output.

Disclosures: M.W. Shiflett: None. **T.S. Tran:** None. **M. Gavin:** None.

Poster

531. **Neural Mechanisms Associated with Autistic Behaviors in Animals**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 531.20/Q8

Topic: D.06. Eye Movements

Title: Visual preference for images of humans in non-human primates; relevance to primate models of autism

Authors: D. DZIOBEK[1], S. ZHANG[2], J. ASHE[1], *X. LU[3];
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Univ. of Minnesota, Biomedical Engineering, MN; [3]Brain Sci. Ctr., VA Med. Center, Minneapolis, Minneapolis, MN

Abstract: Autism is a devastating neurological disorder of unknown cause, unclear pathogenesis and without an effective treatment that appears to be increasing in prevalence. Although there are many animal models of this disorder particularly in rodents, the relevance of these models to a human disease that is defined by abnormal social interaction is debatable. In an effort to characterize natural social behaviors in the non-human primate that might be targeted in developing a primate model of the disorder, we studied preferences for different classes of visual images: (i) neutral non-animate objects, (ii) images of familiar foods, and (iii) images of human faces. We trained non-human primates on a task in which they were presented two visual images simultaneously, and are asked to choose between them. First they were required to hold their gaze on a central fixation point for 500-700 ms after which were presented two images located at either 0 degree (right) and 90 degree (up) positions, or at 180 degree (left) and 270 degree (down) positions but maintained fixation on the center point for another 500-700 ms (delay). After this delay time, the center fixation point disappeared (Go signal) and the subject made a saccade to one of the images and to hold on the image target for 300 ms to receive the reward. The eye movements were monitored continuously and the subjects were rewarded regardless of

which image they chose. We found that, when presented with two neutral inanimate images (e.g. two different chairs), the monkey showed no preference (51% vs. 49%). When images of a human face was paired with either a neutral object (e.g. palm tree or chair) and a familiar food (e.g. banana or peanuts), the monkey preferred the face in more than 90% of trials. There were no clear differences in preferences for familiar (experimenters) or unfamiliar (George W. Bush) faces relative to the other classes. Furthermore, in addition to choice preference, the animals had shorter reaction time and eye movement time to human faces. Our data suggest that the preference of non-human primates for human faces, even when the competing object was a familiar food, is driven by considerations of social cognition that are so prominent in humans. We believe that this natural tendency of non-human primates to prefer images of other primates could be used to probe autistic behavior in monkey models of autism.

Disclosures: D. Dziobek: None. **S. Zhang:** None. **J. Ashe:** None. **X. Lu:** None.**Poster**

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.01/Q9

Topic: C.07. Developmental Disorders

Title: Reexpression of Mecp2 in GABAergic neurons is sufficient to rescue the effects of global Mecp2 deletion

Authors: *K. M. URE, H. LU, E. SZTAINBERG, H. ZOGHBI;
Baylor Col. of Med., Houston, TX

Abstract: Rett syndrome is a devastating neurological disorder affecting predominantly young girls. It is characterized by the loss of acquired milestones, including language, and the development of repetitive hand movements, ataxia, breathing abnormalities, seizures, and autistic-like features. The disease is caused by mutations in MECP2, which encodes a chromatin remodeling protein that binds methylated CpGs. Mecp2-null male mice and heterozygous female mice replicate most of the phenotypes seen in human patients. Interestingly, deletion of Mecp2 only from neurons expressing vesicular inhibitory amino acid transporter (Viaat), a specific marker of inhibitory neurons, replicates most of the clinical symptoms of Rett, including ataxia, repetitive behaviors, breathing abnormalities, and learning and memory deficits, likely due to reduced GABA signaling. This finding highlights the critical role of proper GABAergic neuronal function in the pathogenesis of Rett syndrome and points to the inhibitory circuitry as a potential target for therapeutic intervention. To test this, we genetically reactivated Mecp2 expression in Viaat-expressing neurons using a conditional allele of Mecp2 engineered to contain a flox-stop-flox locus upstream of the Mecp2 coding region, resulting in the generation of mice that express

MeCP2 exclusively in GABAergic neurons. These animals were evaluated along with all relevant controls (Mecp2 null, Viaat-Cre alone, and wildtype). Both male and female “rescue” mice showed normalized body weight and improvement in ataxia, apraxia, learning and memory, and social interaction, along with significantly extended lifespan and some inhibitory signaling normalization. However, the rescue mice did not exhibit a full rescue of all Mecp2-deletion phenotypes; they had noticeable tremor, sensorimotor gating defects (as measured by prepulse inhibition), and died prematurely, with 50% of male rescue mice dying by 40 weeks of age. Furthermore, there was some loss of behavioral benefits later in life. These findings suggest that modulation of GABAergic function in the Rett brain may be a viable therapy option, particularly at early stages of the disorder; to this end we have begun pharmacological studies in the mouse model of Rett syndrome to determine the extent of benefit from enhancing GABAergic signaling.

Disclosures: K.M. Ure: None. **H. Lu:** None. **E. Sztainberg:** None. **H. Zoghbi:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.02/Q10

Topic: C.07. Developmental Disorders

Support: IRSF Research Grant #2906

Title: Rett Syndrome like phenotypes in the R255X Mecp2 mutant mouse are rescued by MECP2 transgene

Authors: *M. R. PITCHER[1,2], A. FISHER[3], N. C. SCHANEN[3], J. L. NEUL[1,2];
[1]Baylor Col. of Med., Houston, TX; [2]Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX; [3]Nemours Biomed. Res. Dept., Wilmington, DE

Abstract: Over thirty percent of Rett Syndrome (RTT) cases are due to nonsense mutations in *MECP2*, where a change in nucleotide sequence leads to a premature stop codon in the mRNA transcript. One strategy to overcome disease-causing stop mutations is treatment with nonsense suppressing read-through compounds, such as gentamicin, which reduce the stringency and fidelity of ribosomes translating mRNA messages to allow expression of full length proteins from a mutated gene. To determine if this strategy may be useful in RTT we characterized a new mouse model of RTT that has a knock-in nonsense mutation (p.R255X) in the *Mecp2* locus (*Mecp2*$^{tm1.1Irsf/J}$). *Mecp2* is a four exon gene that encodes two functional domains: the methyl binding domain from exons 3 and 4 and the transcription repression domain in exon 4. Because

the R255X mutation is located in the transcription repression domain of *Mecp2*, it is possible that a dominant negative DNA binding truncation product could be produced from the disease allele. To determine if the truncated gene product acts as a dominant negative allele, we genetically introduced an extra copy of *MECP2* via a *MECP2* transgene. This allows us to determine whether adding a wild-type version of MeCP2 is sufficient to rescue phenotypic abnormalities in *Mecp2*$^{tm1.1Irsf/J}$ mice, or whether the truncated allele has a dominant negative effect insurmountable by a wild-type copy. *Mecp2*$^{tm1.1Irsf/J}$ mice have phenotypes nearly identical to complete null animals: decreased weight early in life, decreased heart rate late in life, abnormal breathing phenotypes, poor motor coordination, and decreased survival time. The addition of the *MECP2* transgene to *Mecp2*$^{tm1.1Irsf/J}$ mice abolished the phenotypic abnormalities and resulted in near complete rescue. This provides a proof of concept that this mutation is amenable to read-through therapy. Future studies will include pharmacokinetic and efficacy preclinical trials in the R255X model using read-through compounds that are currently in clinical trials for peripheral indications. We hope to demonstrate that read-through therapy is a viable treatment option for neurological disease caused by nonsense mutations.

Disclosures: M.R. Pitcher: None. **N.C. Schanen:** None. **J.L. Neul:** None. **A. Fisher:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.03/Q11

Topic: C.07. Developmental Disorders

Support: Start-up funds from Midwestern University

Title: A comparison of cortical and cerebellar gene dysregulation in three MeCP2 mutant mouse models

Authors: *G. M. JENTARRA, B. CHAVIRA;
Biochem., Midwestern Univ., Glendale, AZ

Abstract: Mutations in the MeCP2 gene, encoding methyl-CpG binding protein 2, are associated with the neurodevelopmental disorders Rett syndrome and X-linked mental retardation (XLMR). This gene has various functional domains including the N-terminal methyl-CpG-binding domain (MBD) and the C-terminal transcriptional repressor domain (TRD). MECP2 is believed to act as both a transcriptional activator and a repressor of the expression of other genes. In order to explore the results of compromised or lost function of one or both of these domains, gene expression in three MeCP2 mutant mouse models was examined using pathway specific qPCR

array plates. The array chosen examined the expression of genes involved in forming the receptors for the major neurotransmitters in the brain. The MeCP2 mutant models used included the A140V model, which replicates a MeCP2 point mutation in the MBD associated with XLMR in males; the 308/y model, which has a C-terminal truncation deleting the TRD of MeCP2; and the "Bird" MeCP2-null model which does not produce functional MECP2 protein. Arrays were run on both cortical and cerebellar brain tissue from 6 week old male mutant mice and their wild type littermates. The data indicate that genes involved in forming the receptors for GABA, acetylcholine, and dopamine are commonly dysregulated due to MeCP2 mutations. These neurotransmitters have been previously implicated in Rett syndrome. In addition, the study identified dysregulation of genes encoding receptors for tachykinin, neuropeptide Y, somatostatin and serotonin. The results of this study demonstrated substantial changes in gene expression which varied based on the brain region sampled (cortex versus cerebellum) and on the specific mutation.

Disclosures: G.M. Jentarra: None. **B. Chavira:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.04/Q12

Topic: C.07. Developmental Disorders

Support: 2010CB945202

2012CB966303

90919057

Title: Circuitry-dependent and independent phenotypes of MeCP2 deficient human neurons derived from ESCs and Rett Syndrome specific iPSCs

Authors: *X. CHEN[1,3], X. HAN[4], B. BLANCHI[2], W. GUAN[4], L. CHENG[5], X. ZHANG[3], Y. YU[4], Y. E. SUN[1,3,2];
[1]Pharmacol., [2]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA; [3]Stem Cell Res. Ctr., Tongji Univ., Shanghai, China; [4]Inst. of Brain Sci. and State Key Lab. of Med. Neurobio., Shanghai, China; [5]Tongji Hosp., Shanghai, China

Abstract: It becomes increasingly recognized that changes in synaptic transmission and/or neural network properties are regional- and circuitry-specific with various neurological

conditions. This feature poses a huge challenge for using patient-specific induced pluripotent stem cells (hiPSCs) to model neurological disorders, because currently little could be done to control the context of the various neural network formed in such “disease-in-a-dish” models. Here, we report that neurons derived from human embryonic stem cells (hESCs) and Rett syndrome (RTT) isogenic hiPSCs exhibit variable spontaneous postsynaptic currents and variable ratios between excitation-inhibition from culture to culture. Intentional alterations of neural circuitry contents using a ventralizing agent changed neural transmission phenotype, suggesting a circuitry-specific nature of such phenotype. In contrast, cell-intrinsic electrophysiological and morphological properties of RTT neurons demonstrate consistent changes, independent of the type of circuitries formed.Our findings demonstrate that “disease-in-a-dish” models using hiPSC-derived neurons, while may capture cell-intrinsic phenotypes of diseased neurons, could be rather challenging for assessing neural-circuitry-dependent phenotypes.

Disclosures: X. Chen: None. **X. Han:** None. **B. Blanchi:** None. **W. Guan:** None. **L. Cheng:** None. **X. Zhang:** None. **Y. Yu:** None. **Y.E. Sun:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.05/Q13

Topic: C.07. Developmental Disorders

Support: NIH Grant HD045022

NIH Grant R37-CA084198

SFARI

NARSAD

IRSF

Title: Genetically engineered human pluripotent stem cell model of Rett Syndrome

Authors: *Y. LI, R. JAENISCH;
Whitehead Institute, MIT, Cambridge, MA

Abstract: The advent of human pluripotent stem cell technology, including that of embryonic stem cells and induced pluripotent stem cells, has opened up a new avenue in human disease

modeling. A crucial limitation in using these patient-specific pluripotent stem cells is the lack of genetically-matched controls. Here we report the generation of isogenic human pluripotent stem cell model of Rett Syndrome by genetically modifying the MECP2 gene. Using TALEN-mediated gene targeting, we have introduced loss-of-function mutation into the MECP2 gene, and generated hemizygous, heterozygous and homozygous null cells. These isogenic human pluripotent stem cells were differentiated into neural progenitor cells as well as mature neurons and astrocytes. We showed that neurons derived from MECP2 null pluripotent stem cells have smaller soma and reduced neurite arborization compared to their isogenic controls. Further analyses revealed that MECP2 null neurons bare key molecular, cellular and physiological features of Rett Syndrome. In summary, our results demonstrate the feasibility of TALEN-mediated gene targeting in generating an in vitro model of human neurodevelopmental disorder. By dissecting disease-related phenotypes in MECP2 null neurons, we hope to investigate disease mechanism, and develop therapeutic strategies that ameliorate these phenotypes in vitro and in vivo.

Disclosures: Y. Li: None. **R. Jaenisch:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.06/Q14

Topic: C.07. Developmental Disorders

Support: Association Française contre les Myopathies (AFM)

Title: *In vivo* testing of a self-complementary AAV9 construct expressing a codon-optimized Mecp2 transgene in a preclinical model of Rett Syndrome

Authors: *V. MATAGNE, L. VILLARD, J.-C. ROUX;
INSERM UMR_S910 - Aix-Marseille Univ., Marseille, France

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder primarily affecting CNS functions but also peripheral functions. There is currently no cure for the disease and treatments available are aimed at improving RTT symptoms. Most RTT cases are due to mutation in methyl CpG binding protein 2 (MECP2), whose main function is that of a global transcriptional repressor.
The recent findings that reactivation of Mecp2 rescued adult diseased RTT mice not only indicates that MECP2 is needed for normal adult function (Robinson et al, Brain 2012) but also that gene therapy might be beneficial for RTT patients, even after the disease has started. Proof

of principle that gene therapy was beneficial in a RTT mouse (Mecp2-null mice) has been recently reported by Gadalla et al (Mol Ther, 2013). The authors showed that benefits of the treatment were highest when the virus was directly administered in the brain of newborn mice, while a more translational approach (i.v. injection in young adult Mecp2-null sick mice) yielded minimal improvement.
In order to try and improve vector delivery and expression, we designed two plasmid constructs expressing GFP (control virus) or a codon-optimized version of Mecp2 (termed MCO) under the regulation of the mouse Mecp2 promoter (pMecp2) and confirmed their efficacy in vitro by transfecting NIH/3T3 mouse fibroblasts. Western blot showed that transfection with the MCO plasmid resulted in a twofold increase in MECP2 protein expression compared to cells transfected with a plasmid expressing the endogenous mouse Mecp2e1 gene under the control of a CMV promoter (6.45±1.1 for MCO vs 3.4±0.8 for Mecp2e1, in arbitrary units, ImageJ densitometric analysis). AAV9 viruses carrying either plasmid constructs (pMecp2-GFP or pMecp2-MCO) were produced and their efficacy was tested in primary mixed neurons/glia cultures from male Mecp2-null mice and their littermate controls. DIV10 primary cultures were infected at a multiplicity of infection (MOI) of 10,000:1 and infected cells were identified by immunocytochemistry. Results showed that the pMecp2 promoter directed the expression of GFP or MCO mostly in neuronal cells (TUJ1+ cells).
As reported by other groups, we found that the AAV9 control virus crosses the BBB after i.v. injection (through the tail vein) in wild-type mice and principally transduce neuron-like cell types throughout the brain. Our next experiments will determine whether administration of the scAAV9-MCO virus to a RTT mouse model (Mecp2-null mice) is able to rescue the neurological symptoms seen in these mice.

Disclosures: V. matagne: None. **L. Villard:** None. **J. Roux:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.07/Q15

Topic: C.07. Developmental Disorders

Support: National Natural Science Fond of China 81100848

Title: Interneuron-specific mecp2 reactivation rescues rett syndrome phenotypes

Authors: ZHOU[1], W. WU[2], X.-M. LI[3];
[1]Inst. of Neuroscience, Zhejiang Univ., Zhejiang, China; [2]The children's hospital, Zhejiang Univ. Sch. of Med., Hangzhou, China; [3]Inst. of Zhejiang Univ., Hangzhou, China

Abstract: Rett syndrome (RTT) is considered as a neurodevelopmental disorder caused by mutation of X-linked *MeCP2* gene which encodes methyl CpG binding protein 2 (MeCP2) that regulates gene transcription. MeCP2 null or conditional mutant mice exhibit many aspects of Rett syndrome phenotypes. However, the underlying mechanism is still to be uncovered. Here we reactivated the MeCP2 in GABAergic neurons specifically by crossing MeCP2-Stop mice with Dlx5/6-Cre mice. Mice with the reactivated MeCP2 showed improvement in body weight, life span, locomotor ability, coordination, anxiety, social ability, and learning and memory compared to non-rescue MeCP2-Stop mice. In conclusion, GABAergic neurons play an important role in RTT pathogenesis by regulating the expression of some genes that may contribute to the development of new therapy for RTT.

Disclosures: Zhou: None. **W. Wu:** None. **X. Li:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.08/Q16

Topic: C.07. Developmental Disorders

Support: NSF Neuroengineering IGERT Fellowship

Title: VEGF inhibition as a potential treatment for FXS neocortical vasculature abnormalities

Authors: *A. BELAGODU, R. GALVEZ;
Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Fragile X syndrome (FXS) is the most common form of inherited mental retardation affecting roughly 1:3,600 males and 1:8,000 females. FXS is primarily caused by the transcriptional silencing of the *FMR1* gene which encodes for Fragile X Mental Retardation Protein (FMRP). Recent analyses from our laboratory have demonstrated abnormal vasculature density in a FXS mouse model (Galvan & Galvez 2012). One of the most prominent regulators of vasculature grow is the vascular endothelial growth factor (VEGF). VEGF expression has been shown to be directly correlated to the amount of vasculature. Various behavioral manipulations that increase brain blood vessels, such as enriched rearing and exercise, also increase VEGF expression. Likewise pharmacologically decreasing VEGF expression results in

decreased vasculature. These analyses strongly suggest that altered VEGF expression is a possible cause for the abnormal FXS vasculature phenotype. In support of this assertion our recent preliminary data has demonstrated that VEGF is over-expressed in the neocortex of adult FXS mice. To potentially alleviate this FXS vasculature phenotype the following study used the VEGF antagonist, bevacizumab. Bevacizumab is an anti-cancer medication that has been used to decrease vasculature on tumor cells. It is a humanized monoclonal antibody which binds to VEGF-A and all its isoforms, preventing it from binding to the VEGF receptor. To block VEGF activity 5mg/kg of bevacizumab was injected IP into PND 60 and 11 month old FXS and wild type (WT) mice every other day for 10 days. Blood vessel density in the visual cortex was then examined. Our findings demonstrated that bevacizumab could be used to reduce neocortical vasculature and as a potential treatment for the vasculature phenotype in FXS. Subsequent analyses will need to determine the cognitive and behavioral implications of bevacizumab treatment in FXS.

Disclosures: **A. Belagodu:** A. Employment/Salary (full or part-time):; NSF Neuroengineering IGERT Fellowship. **R. Galvez:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.09/Q17

Topic: C.07. Developmental Disorders

Support: NIH Grant NS078753

Angelman Syndrome Foundation

Autism Speaks

Title: Electrophysiological characterization of iPSC-derived neurons from Angelman syndrome and Dup15q autism patients

Authors: ***J. J. FINK**, K. A. BOLDUC, T. M. ROBINSON, E. S. LEVINE;
Neurosci., Univ. of Connecticut Hlth. Ctr., Farmington, CT

Abstract: Individuals with a deletion of chromosome 15q11-q13 suffer from Angelman syndrome (AS), a neurogenetic developmental disorder characterized by intellectual disability, ataxia, absent speech, and seizures. The specific gene that is responsible for AS encodes the ubiquitin protein ligase UBE3A. Interestingly, individuals with a duplication of the same

chromosomal region suffer from a form of autism known as 15q duplication syndrome (dup15q). In both syndromes, alterations in synaptic signaling and plasticity appear to play a critical role in the disease phenotype, but the relevant downstream targets of UBE3A are unknown. The discovery of genomic reprogramming of human somatic cells into induced pluripotent stem cell (iPSC) lines provides a novel way to model human diseases with complex genetics. We are using electrophysiological approaches to examine synaptic activity and intrinsic properties of iPSC-derived neurons from AS and dup15q patients, as well as control subjects. After six weeks of in vitro development, a low level of spontaneous synaptic activity was seen in neurons from all genotypes, with no significant difference in the frequency or amplitude of synaptic events across genotype. However, by twelve weeks in vitro, control neurons displayed a dramatic increase in the frequency of synaptic events, which was significantly greater than the frequency in both AS-derived and dup15q-derived neurons, suggesting an increase in synapse number and/or release probability in the control neurons. There was no significant difference in mean amplitude across genotype. We also examined activity-dependent synaptic plasticity using a protocol to enhance NMDA receptor activation and increase cAMP levels. In neurons derived from a control subject, 7/10 cells showed a long-term increase in the frequency of spontaneous synaptic events, with no change in mean amplitude. We are currently exploring potential differences in synaptic plasticity in AS and dup15q-derived neurons. Overall, these approaches may prove useful for identifying novel targets for drug discovery and for screening potential therapeutics aimed at reversing the language and cognitive impairments as well as the seizures and movement disorders associated with Angelman syndrome and autism.

Disclosures: J.J. Fink: None. **K.A. Bolduc:** None. **T.M. Robinson:** None. **E.S. Levine:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.10/Q18

Topic: C.07. Developmental Disorders

Support: NIGMS - T32GM008307

NINDS - 1 F31 NS077621-01

Title: Genetic studies to gain insight into the function of the MeCP2 domains *in vivo*

Authors: *L. HECKMAN[1,2], H. Y. ZOGHBI[1,2,3];
[1]Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX; [2]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [3]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: Rett syndrome (RTT) is a debilitating neuropsychiatric disorder caused by mutations in the X-linked methyl-CpG binding protein 2 gene, which encodes for a protein with the same name (MeCP2). Many of the RTT-causing mutations create a truncating null allele, suggesting that RTT is due to a loss of MeCP2 function. Interestingly, overexpression of MeCP2 due to duplications in Xq28 also causes a progressive neurological syndrome that shares many features with RTT. Studies in mice that either lack (RTT model) or express 2X MeCP2 (duplication model) reveal that the loss and gain of MeCP2 has opposing effects on excitatory synapses and gene expression, suggesting that the duplication causes the disorder by a "hyperfunction" mechanism. However, the challenge has been to understand exactly what function of MeCP2 is being exaggerated when it is overexpressed. Traditionally, MeCP2 was believed to be a transcriptional repressor, yet gene expression data from animal models does not support this model. Newer hypotheses proposed that MeCP2 dampens transcriptional noise, such that in its absence, increased basal transcription leads to decreased expression of neuronal genes. This model, however, does not quite explain why doubling the protein will enhance expression of activity dependent neuronal genes. Another hypothesis proposes that overexpression of MeCP2 might titrate co-repressors, thus resulting in an increase of gene expression. To determine if DNA binding by the methyl-CpG-binding domain (MBD) is necessary for the neuronal dysfunction resulting from MeCP2 overexpression, I have generated mice that overexpress MeCP2 alleles with three different RTT-causing mutations: T158M, which reduces methyl-CpG binding, R111G, which abolishes methyl-CpG binding without affecting the structure of the MBD, and R306C, which lies within the transcriptional repression domain (TRD). Transgenic mouse lines that express each allele at levels similar to endogenous levels have been generated thus allowing me to study if doubling MeCP2 levels with an allele that either compromises DNA binding activity or one that is beyond the MBD are consequential. Behavioral, physiological and molecular studies of these alleles are ongoing both in the context of overexpression and in a MeCP2 null background. These models promise to provide new insight into the mechanism by which MeCP2 overexpression leads to neurological dysfunction.

Disclosures: L. Heckman: None. **H.Y. Zoghbi:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.11/R1

Topic: C.07. Developmental Disorders

Support: NIH Grant MH094449

Title: Characterization of cortical neuron defects in Fragile X mice

Authors: *A. AHARON, Y. ZUO;
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Abstract: Fragile X syndrome (FXS) is the most common form of genetically inherited mental retardation. It is caused by the transcriptional silencing of the FMR1 gene that encodes for fragile X mental retardation protein. One hallmark of disease is the abundance of immature dendritic spines in cortical pyramidal neurons. This abnormality has been found in various brain regions in the fixed tissue of human FXS patients, as well as in FMR1 knock out (KO) mice (a mouse model of FXS). So far, most studies concerned with the spine phenotype in FXS have been focused on adult knockout mice, while heterozygous mice continue to go unstudied. Furthermore, it remains unclear how this abnormal spine phenotype progresses during the course of development, and exactly how different neuronal cell types are affected in the diseased brain has yet to be discovered. In this study, we set out to explore these questions by crossing FMR1 KO mice with thy1-GFP-M line mice, a line of mice sparsely labeling individual pyramidal neurons with green fluorescent protein. We then analyzed the neuronal morphology and spine density in layer V pyramidal neurons in the cortex of the wild type (WT), FMR1 heterozygous and FMR1 KO mice. We found that, at 1 month of age, the spine density of apical dendrites is comparable between FMR1 KO, heterozygous mice and their WT littermates. However, FMR1 KO and heterozygous mice did not show normal spine pruning during adolescent development, resulting in a significantly higher (~60%) spine density compared to WT mice at 4 months of age. Currently we are investigating more developmental ages to determine when spine abnormality first appears, we are also studying the variation in different cortical regions and cell types.

Disclosures: A. Aharon: None. **Y. Zuo:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.12/R2

Topic: C.07. Developmental Disorders

Support: NIDS Grant NS073875

International Rett Syndrome Foundation

Title: Mecp2-null mice displayed possible endogenous compensatory mechanisms in defective neurotransmitter systems

Authors: *M. F. OGINSKY, W. ZHONG, C. M. JOHNSON, N. CUI, C. JIANG;
Biol., Georgia State Univ., Atlanta, GA

Abstract: Rett syndrome (RTT) is an autism-spectrum disorder caused by mutations to the X-linked gene, methyl-CpG binding protein 2 (MeCP2). MeCP2 binds to methylated DNA to regulate transcription. Knockout of this gene in mouse models leads to abnormalities in dendritic formation, intrinsic membrane properties, synaptic transmission and neurotransmitter-synthesizing enzyme expression, which may underlie the development of RTT. Although many systems are affected by mutations in the *MECP2* gene, it is possible that the body may develop certain compensatory mechanisms to alleviate the abnormalities. The norepinephrine (NE) system originating mostly in the locus coeruleus (LC) is defective in RTT and *Mecp2*-null mice. LC neurons are NE-ergic and are subject to modulation by GABA and Acetylcholine (Ach). The relationship among these modulators provides a good system to test our potential compensatory hypothesis. Therefore, we performed studies of neurons in the LC area in WT and *Mecp2*-null mice. The $GABA_A$-ergic IPSC frequency was attenuated by a nicotinic receptor antagonist and this attenuation was greater in *Mecp2*-null mice. The amplitude did not change suggesting presynaptic alterations in nicotinic receptors. With tissue micropunctures from the LC region, the expression of nicotinic receptors was analyzed with qPCR. The largest nicotinic receptor expression levels were for the α3 and α4 subunits in wild-type littermates. The expression of these subunits was decreased by >40% in *Mecp2*-null mice. The α7 and β2 nicotinic receptors subunits that were expressed in low levels in the wild-type were upregulated in the *Mecp2*-null mice. These data suggest that although the *Mecp2* disruption causes a decrease in α3 and α4 subunits, LC neurons appear to be able to compensate for the defects by increasing α7 and β2 expression. This may lead to an increase in $GABA_A$-ergic synaptic transmission that is deficient in *Mecp2*-null mice. The presence of such endogenous compensatory mechanisms may allow novel medical intervention to prevent advancement of the disease.

Disclosures: M.F. Oginsky: None. **W. Zhong:** None. **C.M. Johnson:** None. **N. Cui:** None. **C. Jiang:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.13/R3

Topic: C.07. Developmental Disorders

Support: Erik Haferkamp Memorial Scholarship

Title: Neocortical developmental vasculature abnormalities in a mouse model of fragile X syndrome

Authors: S. A. FLEMING, *R. GALVEZ;
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Abstract: Fragile X Syndrome (FXS), the most common form of inherited mental retardation, is caused by the transcriptional silencing of the fmr-1¬ gene, which codes for the Fragile X Mental Retardation Protein. The syndrome is accompanied by intellectual disability, autistic-like behaviors, and a variety of physical abnormalities. The most prominent neuronal abnormality is the abundance of long, thin, immature dendritic spines. Studies have also demonstrated that boys with FXS have abnormal cerebral blood flow. Furthermore, recent analyses from our laboratory have demonstrated a lack of age-induced blood vessel density (BVD) plasticity in middle-aged mice with FXS (Galvan & Galvez 2012). During development neocortical regions establish neuronal connections necessary for normal cognition and neuronal function. Abnormal vasculature growth during these critical developmental periods would alter proper neuronal development causing or exacerbating many of the neuronal and cognitive abnormalities associated with FXS. The following study set out to characterize BVD in the visual neocortex of mice with FXS during development. Wildtype (WT) and FXS mice were sacrificed at postnatal dates 10, 20, 35, 60. Neocortical sections were then stained for collagen IV, a protein found in the lumen of blood vessels. Once stained, stereological techniques were used to characterize BVD in the visual neocortex of FXS and WT mice. Our findings demonstrate that FXS exhibit abnormal BVD compared to WT mice during development. Such analyses will provide vital insight into factors contributing towards the anatomical and cognitive abnormalities observed in FXS.

Disclosures: S.A. Fleming: None. **R. Galvez:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.14/R4

Topic: C.07. Developmental Disorders

Support: NIH Grant HD062553

Title: Roles of MeCP2 in the autonomic nervous system

Authors: *T.-W. HUANG[1], J. L. NEUL[2];
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[2]Departments of Pediatrics - Neurol., Baylor Col. of Med., Houston, TX

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder associated with loss of communication and purposeful hand skills as well as several autonomic deficits. These autonomic problems may contribute to the sudden death observed in a fraction of people with RTT. Mutations in the gene encoding Methyl-CpG-binding protein 2 (MECP2) cause 95% of RTT cases, and mice lacking MeCP2 function exhibit the pathological features similar to RTT patients.

Removing MeCP2 from brainstem and spinal cord in mice causes early lethality and autonomic phenotypes including decreased heart rate and abnormal respiratory response to hypoxia. In addition, re-expressing MeCP2 within the region is sufficient to rescue these phenotypes. The brainstem is known to contain neural circuits critical for the regulation of autonomic function. To determine which neuronal circuits require MeCP2 for normal hypoxic respiratory response and survival, Cre/LoxP system is used to remove or re-express MeCP2 in specific regions that are known to be important for the control of breathing.

Using transgenic mice express Cre recombinase in the HoxA4 domain; MeCP2 is removed from the caudal medulla, spinal cord, and peripheral nervous system (PNS). The conditional knockout mice showed abnormal motor functions and early lethality, but had normal heart rate and hypoxic breathing response. In addition, MeCP2 expression solely within the HoxA4 domain is sufficient for the survival of rescued animals. To examine whether restored MeCP2 expression in PNS is related to the rescued phenotypes, Sox10-Cre transgenic line was used to restore MeCP2 expression in PNS and oligodendrocytes. Animals expressing MeCP2 in the Sox10 domain continued to have poor motor coordination, abnormal breathing, and early lethality. The results suggested that MeCP2 expression in caudal medulla and spinal cord is critical for the motor coordination and survival, but MeCP2 function within the PNS or oligodendrocytes isn't. MeCP2 expression in HoxA4 or Sox10 domain did not rescue the respiratory hypoxic response. To further determine the regions require MeCP2 for the breathing control, MeCP2 expression was restored in two anatomical regions known to be important nodes for the chemoreflex breathing control circuit, the nucleus tractus solitarii (NTS) and the retrotrapezoid nucleus (RTN), by using Phox2b-Cre transgenic line. Phox2b-Cre rescued animals showed normal breathing response to hypoxia but still display early lethality. These results indicate that MeCP2 expression in NTS and RTN is important for the chemoreflex pathway, and suggest that abnormal breathing response to hypoxia may not relate to the early lethality.

Disclosures: T. Huang: None. **J.L. Neul:** None.

Poster

532. Rett's, Fragile X, and Angelman's Disorders

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.15/R5

Topic: C.07. Developmental Disorders

Support: 973 Program Grant 2011CBA00400

NRSF 30925016

NRSF 31021063

Title: Palmitoylation-dependent cdkl5-psd95 interaction regulates synaptic targeting of cdkl5 and synaptic transmission

Authors: *Z.-Q. XIONG, Y.-C. ZHU, D. LI;
Inst. Neurosci, Shanghai, China

Abstract: The X-link gene cyclin-dependent kinase-like 5 (CDKL5) is mutated in patients with severe neurodevelopmental disorders including an early-onset variant of Rett syndrome. The expression of CDKL5 is developmentally regulated and enriched in the brain. Our recent studies have shown that CDKL5 plays important roles in neuronal development such as neurite growth and dendritic spine morphogenesis. The postsynaptic scaffolding protein PSD-95 is a major palmitoylated protein in neurons and its synaptic targeting depends on palmitoylation. We find that PSD-95 interacts with CDKL5 in a palmitoylation-dependent fashion and regulates its targeting to excitatory synapses. Interestingly, certain disease-associated mutations which abolish this interaction reduce synaptic localization of CDKL5. Moreover, disruption of this interaction diminishes synaptic targeting of CDKL5 and inhibits dendritic spine growth. Thus, our findings reveal an unexpected role of palmitoylated PSD-95 in synaptic targeting of CDKL5, which may represent a novel mechanism for protein trafficking. These results may also provide insights into the pathogenesis of CDKL5-related disorders.

Disclosures: Z. Xiong: None. **Y. Zhu:** None. **D. Li:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.16/R6

Topic: C.07. Developmental Disorders

Support: RO1 MH085617

P30HD024064

Title: Single-molecule imaging of PSD-95 mRNA translation in dendrites reveals its dysregulation in a mouse model of fragile X syndrome

Authors: *M. F. IFRIM, G. J. BASSELL;
Cell Biol., Emory Univ., Atlanta, GA

Abstract: Fragile X syndrome (FXS) is caused by the loss of the fragile X mental retardation protein (FMRP), an RNA binding protein that regulates translation of numerous target mRNAs. Deficiency of FMRP in a mouse model of FXS, leads to increased basal translation rates of many FMRP target mRNAs, yet there is a loss of group 1 metabotropic glutamate receptor (mGlu)-stimulated translation. Since several FMRP target mRNAs are known to be localized to dendrites, FMRP is hypothesized to regulate dendritic protein synthesis.
We have previously used biochemical methods applied to synaptoneurosomes to demonstrate a role for FMRP in regulating the translation of PSD-95 mRNA. However, PSD-95 mRNA translation within dendrites has not been directly visualized and the extent of translational dysregulation in Fmr1 KO neurons, and particularly in dendrites, has not been examined.
We performed single-molecule analysis of PSD-95 mRNA translation in dendrites of primary hippocampal neurons using a Venus fluorescent protein-based translation reporter with PSD-95 ORF, 5'UTR and 3'UTR.
We show that PSD-95 mRNA translation is predominantly sporadic and occurs in dendrites and dendritic spines. In WT mouse hippocampal neurons, the rate of PSD-95 mRNA translation in dendrites is rapidly increased following mGlu stimulation with DHPG. By contrast, in Fmr1 KO hippocampal neurons, basal rate of PSD-95 mRNA translation was increased and mGlu-stimulated translation was occluded.
We show that PSD-95 mRNA translation is regulated by elements present in both PSD-95 mRNA 5'UTR and 3'UTR. The data obtained from this cell-based assay corroborate and extend earlier findings obtained with synaptoneurosomes by visualizing dysregulated PSD-95 mRNA translation in dendrites from Fmr1 KO neurons and by identifying the role of 5'UTR in PSD-95 mRNA translation regulation. The observed dysregulation in the dendritic synthesis for PSD-95, a critical regulator of synaptic structure and function, has important implications for understanding how impairments in the synaptic proteome and its dynamic regulation may underlie alterations in dendritic spine morphology and synaptic plasticity in fragile X syndrome.

Disclosures: M.F. Ifrim: None. **G.J. Bassell:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.17/R7

Topic: C.07. Developmental Disorders

Title: Altered somatosensory barrel cortex refinement in the developing brain of Mecp2-null mice

Authors: *M. MOROTO[1], A. NISHIMURA[1], M. MORIMOTO[1], K. ISODA[1], T. MORITA[1], M. YOSHIDA[1], S. MORIOKA[1], T. TOZAWA[1], T. HASEGAWA[1], T. CHIYONOBU[1], K. YOSHIMOTO[2], H. HOSOI[1];
[1]Pediatrics, [2]Legal Med., Kyoto Prefectural Univ. of Med., Kyoto, Japan

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the methyl-CpG binding protein 2 (MeCP2) gene. In previous studies, monoaminergic dysfunctions have been detected in patients with RTT and in a murine model of RTT, the Mecp2-null mouse. Therefore, the pathogenesis of RTT is thought to involve impairments in the monoaminergic systems. However, there have been limited data showing that the impairment of monoamines leads to early symptoms during development. We used histochemistry to study the somatosensory barrel cortex in the B6.129P2(C)-Mecp2tm1.1Bird mouse model of RTT. The barrel cortex is widely used for investigating largely serotonin (5-HT) signaling-regulated neuronal development. 5-HT levels were measured by high performance liquid chromatography with electrochemical detection (HPLC/EC), and serotonin transporter (SERT) and 5-HT1B receptor mRNAs were measured in the somatosensory cortex, thalamus and striatum on postnatal days (P) 10, P20 and P40. Mecp2-null mice (Mecp2-/y) had significantly smaller barrel fields than in age-matched wild-type controls (Mecp2+/y) on P10 and P40, but the topographic map was accurately formed. Levels of 5-HT, and SERT and 5-HT1B receptor mRNA expression in the somatosensory cortex did not differ significantly between the Mecp2-null and wild-type mice on P10. However, thalamic 5-HT was reduced in Mecp2-null mice. Our data indicate that a lack of MeCP2 may disturb the refinement of the barrel cortex in the early postnatal period. Our results eliminate a role for somatosensory cortical 5-HT in this mechanism, but suggest that a decrease in thalamic 5-HT might be involved in this phenomenon.

Disclosures: M. Moroto: None. **A. Nishimura:** None. **M. Morimoto:** None. **K. Isoda:** None. **T. Morita:** None. **M. Yoshida:** None. **S. Morioka:** None. **T. Tozawa:** None. **T. Hasegawa:** None. **T. Chiyonobu:** None. **K. Yoshimoto:** None. **H. Hosoi:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.18/R8

Topic: C.07. Developmental Disorders

Support: MH50047

Title: Cortical thickness development associated with fragile X syndrome from childhood to early adulthood

Authors: *E.-M. QUINTIN, J. L. BRUNO, M. M. RAMAN, B. JO, S. HALL, A. LIGHTBODY, A. L. REISS;
Psychiatry, Stanford Univ., Stanford, CA

Abstract: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and single gene cause of autism spectrum disorder. FXS is associated with abnormal frontal and temporal brain morphology including the orbitofrontal, medialfrontal, and fusiform gyri (Hoeft et al., 2010) and superior and middle frontal gyri (Bray et al., 2011). Activation of the superior temporal and fusiform gyri during fMRI face and eye-gaze processing tasks is also atypical in FXS (Garrett et al., 2004; Watson et al., 2008). Recently, increased cortical thickness in frontal and temporal areas of individuals with FXS has been associated with lower scores on cognitive measures (Meguid, 2012). We investigated whether cortical thickness of frontal and temporal areas change at different rates for individuals with FXS compared to controls using a longitudinal design.
The study included three groups of female participants: 46 with FXS, 28 with developmental delays of idiopathic origin (DD), and 57 with typical development (TD); and three groups of male participants: 33 with FXS, 31 with DD, and 53 with TD. MRI scans were performed 1 to 3 times for each participant. The total age range including all time points is 10 to 26 years. Images were obtained on a 1.5 Tesla MRI scanner with a spoiled gradient recalled echo sequence. Mean cortical thickness was calculated with Freesurfer 5.0. We performed hierchical linear modeling separately for males and females to estimate cortical thickness trajectories from 10 to 26 years of age and compared the rates of thickness change between groups (FXS vs. TD and FXS vs. DD). Overall, bilateral cortical thinning of most frontal and temporal areas was found for all groups and both sexes. Similar cortical thinning patterns were found for the FXS and TD female groups. Specific group differences include faster rates of cortical thinning for the FXS vs. TD male groups at the left middle frontal and medial inferior frontal gyri (including orbitofrontal cortex); and for the FXS vs. TD female groups at the right fusiform gyrus; and FXS vs. DD female groups at the left medial inferior frontal gyrus. Rates of cortical thinning tended to be slower for

FXS vs. DD male groups at the left and right superior temporal gyrus.
Atypical cortical thinning rates of frontal and temporal areas associated with FXS is consistent with previously reported abnormal brain morphology and activation of those areas. The results of such investigations can potentially provide robust biomarkers to measure the efficacy of specific drug and behavioral interventions since these areas are associated with cognitive and social functioning.

Disclosures: E. Quintin: None. **J.L. Bruno:** None. **M.M. Raman:** None. **B. Jo:** None. **S. Hall:** None. **A. Lightbody:** None. **A.L. Reiss:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.19/R9

Topic: C.07. Developmental Disorders

Title: Diurnal increase of apnea and reduced GAD1 mRNA expression in respiratory nuclei in Mecp2-deficient mice

Authors: *M. NISHIYAMA[1], M. ASANO[2], S. IWASA[1], A. SUZUKI[1], T. WADA[1], H. TAKIGUCHI[1], T. SHIRAKAWA[1];
[1]Dept. of Pediatric Dentistry, Nihon Univ. Sch. of Dent., Tokyo, Japan; [2]Dept. of Pathology, Nihon Univ. Sch. of Dent., Tokyo, Japan

Abstract: Rett syndrome (RTT) is caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). RTT patients exhibit autistic symptoms, breath holding, seizures and *etc*. It has been shown that breath holding in the patients occurs almost exclusively at daytime. *Mecp2*-null mice (*Mecp2* $^{-/y}$) exhibit frequent apnea similar to that seen in RTT, but there is not a report on its diurnal variation and epigenetic mechanisms underlying the respiratory disturbances are unknown.
We evaluated respiratory functions in unrestrained *Mecp2* $^{-/y}$ using a whole body plethysmography at 2, 3, 5, and 7 weeks of age. At 5 and 7 weeks of age, continuous 24-hour measurement of respiration was carried out in *Mecp2* $^{-/y}$ and wild-type male mice (*wild*). When apnea counts were compared between *Mecp2* $^{-/y}$ and *wild*, it became apparent that the counts were much larger in *Mecp2* $^{-/y}$ than *wild* at all ages examined and throughout the 24h period at 5 and 7 weeks of age. The apnea increased significantly during the light phase of 12:12h light-dark environment in *Mecp2* $^{-/y}$ ($p < 0.01$). In contrast, *wild* showed stable respiratory rhythm and no day-night difference was observed in apnea counts.

Because GABAergic neurotransmission has been reported to be impaired in the brain of *Mecp2* $^{-/y}$, we measured glutamate decarboxylase 1 (GAD1) mRNA expression in the respiratory nuclei (nucleus of the solitary tract and ventrolateral medulla) by quantitative RT-PCR, and found that GAD1 mRNA is reduced in these respiratory nuclei of *Mecp2* $^{-/y}$ compared to *wild* at 2 weeks of age ($p < 0.05$). Methylation analysis of CpG islands in the proximal promoter region of GAD1 DNA extracted from the respiratory nuclei revealed differences of the sites of methylated CpG between *Mecp2* $^{-/y}$ and *wild* while most of the CpGs in the GAD1 promoter were unmethylated. These results indicate that the altered GAD1 expression in the respiratory nuclei may be responsible for the appearance of apnea in *Mecp2* $^{-/y}$.

Disclosures: M. Nishiyama: None. **M. Asano:** None. **S. Iwasa:** None. **A. Suzuki:** None. **T. Wada:** None. **H. Takiguchi:** None. **T. Shirakawa:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.20/R10

Topic: C.07. Developmental Disorders

Support: IRSF Grant n. 2814

Telethon Grant n. GGP09196

Title: Rett Syndrome-related genes share common pathogenic pathways

Authors: N. MORELLO[1], R. PIZZO[1], E. CALCAGNO[1], E. AMENDOLA[2], C. GROSS[2], M. SASSOÈ-POGNETTO[1], *M. GIUSTETTO[1];
[1]Dept. of Neurosci., Univ. of Torino, Torino, Italy; [2]European Mol. Biol. Lab. (EMBL), Monterotondo (Rome), Italy

Abstract: Rett syndrome (RTT) is an X-linked progressive neurodevelopmental disorder. RTT is typically characterized by a broad range of deficits including loss of language skills and hand use, cognitive and motor alterations. While most RTT patients show mutations of the X-linked methyl-CpG-binding protein 2 (MECP2) gene, recently a rare RTT-like syndrome was shown to be associated with mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene that is characterized by early onset seizures. Even though patients carrying MECP2 or CDKL5 mutations show peculiar behavioural and cognitive alterations, they share overlapping features including stereotypical hand movements and deficient language acquisition. Recent evidences suggest that RTT neurological symptoms may arise from excitation/inhibition balance

abnormalities in specific neocortical regions which likely stem from dysfunctional interneurons. However, the cellular and molecular determinants of such dysfunctions are largely unknown. In this study, we analyzed several components of the GABAergic system in two murine models of RTT, the Mecp2tm1.1Jae mutants and a newly-generated Cdkl5-KO strain. Using immunofluorescence, we studied whether the lack of MeCP2 or CDLK5 may alter the normal organization of three different populations of GABAergic neurons and the architecture of inhibitory synapses in neocortical circuits. We found that Mecp2 deletion causes an increase of the density of both parvalbumin (PV) and calretinin (CR) interneurons even at early asymptomatic postnatal stages, without affecting somatostatin (SST) interneurons. A similar increase of PV cell density was also present in Cdkl5-KO mice. The CR-positive interneurons showed an abnormal morphology both in Mecp2 and Cdkl5 mutants. Quantitation of immunolabeling showed a significant increase of perisomatic neuroligin-2 NL2-positive synaptic puncta in MeCP2-KO cortices while, surprisingly, no differences were found in the number of VGAT-positive puncta. These results indicate that the expression of this GABAergic postsynaptic adhesion molecule is modulated by MeCP2. To test whether these alterations in the organization of GABAergic connectivity were cell autonomous, we used a Dlx5/6-Cre line to remove MeCP2 from a subset of forebrain GABAergic neurons. These analyses revealed similar phenotypes in conditional mutants and MeCP2 null mice. Our data support the idea that mutations of RTT-related genes share common pathogenic pathways, namely the abnormal organization of GABAergic systems, that may produce excitation/inhibition imbalance in the brain.

Disclosures: N. Morello: None. **M. Giustetto:** None. **R. Pizzo:** None. **E. Calcagno:** None. **M. Sassoè-Pognetto:** None. **C. Gross:** None. **E. Amendola:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.21/S1

Topic: C.07. Developmental Disorders

Support: NCATS grant UL1TR000075

Title: Development of cortical activity in a rat model of Fragile X Syndrome *In vivo*

Authors: *J. BERZHANSKAYA[1], A. S. GORIN[2], M. A. PHILLIPS[1], M. T. COLONNESE[1]; [1]Pharmacol. & Physiol., [2]Engin. and Applied Sci., George Washington Univ., Washington, DC

Abstract: A critical question in the etiology of developmental neurological disorders is the extent to which pre-symptomatic circuit perturbations contribute to disease phenotype. Taking advantage of a newly developed model for FXS in the rat (the Fragile X mental retardation protein 1 mutant (Fmr1KO)), a species for which visual cortical activity development has been carefully matched to human neonates, we are examining the developmental trajectory of spontaneous and visually evoked activity in the unanesthetized visual cortex of Fmr1KO and wild-type Sprague-Dawley rats between birth and adulthood. In control animals, developing visual cortex passes through two clear functional modes with the transition two days before eye-opening (two weeks before term in humans). During the first period, spontaneous retinal waves (or visual stimuli) drive rapid oscillations on a background of network silence. During the second period, cortical active states emerge, leading to continuous background activity and mature-like visual evoked potentials. The cortical activity patterns of Fmr1KO rats were qualitatively similar to wild-type controls during both developmental periods. During the early developmental period, KO rats demonstrated rapid oscillations on a background of network silence. Transition to the late period occurred before eye opening, as in controls, suggesting no appreciable developmental delay. Quantitative analysis of local field potentials (LFP) during wakefulness showed signs of hyper-excitability at P19-20, with an increase in gamma-band power relative to controls. Continuing analysis of spontaneous and visually evoked LFP and spiking activity throughout the depth of cortex will be used to further characterize the developmental trajectory of cortical excitability in Fmr1KO animals.

Disclosures: J. Berzhanskaya: None. **A.S. Gorin:** None. **M.A. Phillips:** None. **M.T. Colonnese:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.22/S2

Topic: C.07. Developmental Disorders

Support: NIH NS045711 (KMH)

T32 NS069562 (KAC)

Title: Selective disruption of mGluR5-Homer interactions mimics multiple phenotypes of Fragile X Syndrome in mice

Authors: *K. A. COLLINS[1], W. GUO[1], S. A. HAYS[1], G. MOLINERO[2], R. PAYLOR[3], P. F. WORLEY[4], K. M. HUBER[1];

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Abstract: Fragile X Syndrome (FXS) results from transcriptional silencing of the Fmr1 gene and is the most common inherited form of intellectual disability and a leading genetic cause of autism. Altered function of the Gq-coupled, Group 1 metabotropic glutamate receptors, specifically mGluR5, occurs in the FXS mouse model, the Fmr1 knockout (KO) mouse. Pharmacological and genetic reduction of mGluR5 reverses many phenotypes of FXS in animal models and mGluR5 antagonists are currently in clinical trials in FXS and autism patients. A mechanism for mGluR5 dysfunction in Fmr1 KO mice is suggested by the findings that mGluR5 is less associated with its scaffolding protein Homer and restoration of mGluR5-Homer interactions rescues many phenotypes of Fmr1 KO mice (Giuffrida et al., J. Neurosci. 2005; Ronesi et al., Nat. Neurosci. 2012). The Homer family of proteins binds to the intracellular C-terminal tail of mGluR5 and forms multi-protein signaling complexes at the postsynaptic density with mGluR5 and its downstream effectors. Disruption of mGluR5-Homer scaffolds leads to abnormal mGluR5 localization, signaling as well as constitutive or overactive mGluR5. We hypothesized that selective disruption of mGluR5 from Homer may be sufficient to mimic many phenotypes of Fmr1 KO mice. To test this hypothesis, we examined mice with a knockin mutation of mGluR5 (F1128R; mGluR5FR; Cozzoli et al., J. Neurosci. 2009) that abrogates binding to Homer to determine if these mice mimic phenotypes of Fmr1 KO mice. Many phenotypes of Fmr1 KO mice are mimicked in the mGluR5FR mice including mGluR5 localization at the synapse, mGluR5-signaling to translation, neocortical hyperexcitability, and open field behavior. Synaptic plasticity and other behavioral phenotypes are currently being examined. These results indicate that disruption of mGluR5-Homer scaffolds is sufficient to mimic multiple phenotypes of Fragile X Syndrome and reveals specific molecular mechanisms of a complex brain disease.

Disclosures: K.A. Collins: None. **W. Guo:** None. **S.A. Hays:** None. **G. Molinero:** None. **R. Paylor:** None. **P.F. Worley:** None. **K.M. Huber:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.23/S3

Topic: C.07. Developmental Disorders

Title: Cellular mechanisms of dopaminergic dysfunction in Angelman syndrome model mice

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Abstract: Increased *UBE3A* dosage has been linked to autism spectrum disorders, while maternal mutations or deletions in *UBE3A* cause Angelman syndrome (AS). AS is a debilitating neurodevelopmental disorder characterized by intellectual disability, ataxia, epilepsy, abnormal sleeping patterns, developmental delay, and a happy disposition. Previous studies suggest that aberrant dopamine transmission may contribute to AS behavioral phenotypes. Consistent with this idea, recent studies in AS model mice indicate that maternal *Ube3a* loss is associated with pathway-specific deficits in dopamine release (Riday et al., 2012). Specifically, AS mice have increased dopamine release in the mesolimbic pathway, but decreased dopamine release in the nigrostriatal pathway. These data indicate that UBE3A protein is a major regulator of proper dopamine signaling. Based on these results, we hypothesize that these opposing, pathway-specific effects are due to cell type-specific synaptic deficits from projection dopaminergic neurons in the Ventral Tegmental Area (VTA) and Substantia Nigra pars Compacta (SNc). Using *in vitro* slice electrophysiology, our preliminary data indicate that changes in dopamine release are linked to differences in intrinsic neuronal excitability within the midbrain in AS model mice. Using this approach we are investigating the mechanism by which UBE3A regulates dopaminergic transmission in AS model mice, with the expectation that this knowledge will guide more rational treatments for dopaminergic dysfunction in individuals with AS.

Disclosures: J. Berrios: None. **B.D. Philpot:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.24/S4

Topic: C.07. Developmental Disorders

Support: NIH Grant MH085802

NEI NRSA 5F32EY020066-03

Title: Novel microRNA-mediated mechanisms regulate brain growth factor expression in Rett Syndrome - Implications for therapeutics

Authors: *N. MELLIOS[1], S. D. SHERIDAN[2], S. KWOK[1], D. FELDMAN[1], B. CRAWFORD[1], J. WOODSON[1], S. HAGGARTY[2], M. SUR[1];

[1]Brain and Cognitive Sci., MIT/ Picower Inst. For Learning and Memory, Cambridge, MA; [2]Harvard Med. School, MGH, Boston, MA

Abstract: Rett Syndrome is a debilitating childhood-onset neurodevelopmental disorder that is predominantly caused by mutations in methyl-CpG-binding protein 2 (MEPC2). Using the MeCP2 knockout (KO) mouse model of the disease we uncovered a novel miRNA-mediated molecular pathway that bridges the observed alterations in Brain-derived neurotrophic factor (BDNF) and Insulin-like growth factor 1 (IGF1) expression in the brain of MeCP2 KO mice. Importantly, chronic treatment with a β-2 adrenergic receptor agonist completely normalized the expression of the components of the affected molecular pathway in the cerebellum of Mecp2 KO mice, and resulted in greatly increased survival, improved respiratory function, social recognition, and motor coordination; all cardinal symptoms of Rett syndrome. Notably, co-administration of the same β-2 adrenergic receptor agonist with recombinant human IGF1, further ameliorated the phenotype of MeCP2 KO mice, and resulted in a notable increase in survival. In parallel we used patient-derived induced pluripotent stem cells (IPSCs) to screen more effectively for miRNAs that are affected in Rett Syndrome. Our results from IPSC-derived neuronal cultures revealed among others an additional miRNA family that is robustly increased in two different patient-derived samples, at two different developmental stages, and following viral-mediated knockdown of WT samples. Protein and RNA expression analysis uncovered altered levels of known targets of the affected miRNA family, which are upstream regulators of BDNF expression. In summary we show using both a mouse and IPSC-model of Rett syndrome that a subset of Mecp2-regulated miRNAs are important effectors of complex regulatory networks related to brain growth factor expression, and reveal novel therapeutic alternatives for the treatment of Rett syndrome.

Disclosures: N. Mellios: None. **S.D. Sheridan:** None. **S. Kwok:** None. **D. Feldman:** None. **B. Crawford:** None. **J. Woodson:** None. **S. Haggarty:** None. **M. Sur:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.25/S5

Topic: C.07. Developmental Disorders

Support: Barrow Neurological Foundation

Title: Molecular regulation of neuronal size in MeCP2 A140V mutant mice

Authors: *S. RANGASAMY, S. OLFERS, V. NARAYANAN;
Developmental Neurogenetics, Barrow Neurolog. Inst., PHOENIX, AZ

Abstract: Rett syndrome (RTT), an X-linked dominant neurological disorder caused by mutation of the MeCP2 gene, is characterized by stereotypical hand movements and autistic features. Pathological studies in mouse models and human cases of Rett syndrome have shown a reduction in neuronal size, dendritic complexity and increased cell packing density. Our laboratory has created a mouse model (MeCP2 A140V "knock-in" mutant) expressing a human MeCP2 mutation linked to an X-linked mental retardation phenotype. In this model, we have identified decreased dendritic branching of cortical layer III pyramidal neurons, and increased cell packing density in several brain regions. Here, we report measurements of neuronal soma and nuclear size in cultured neurons from A140V and wild type (WT) mice. Soma size, measured after staining with beta III tubulin, was reduced by ~30% reduction at 21 days of in vitro (DIV) in mutant hippocampal neurons compared to WT. This reduction in neuronal soma size is observable as early as 3 DIV and is accompanied by reduction in nuclear size. The mammalian target of rapamycin (mTOR) signaling network, consisting of two catalytic subunit complexes mTORC1 and mTORC2, is crucial for neuronal development and long-term modification of synaptic strength. Recent studies have shown that mTORC2 plays an important role in regulation of brain morphology, neuronal size and function. Loss of Rictor protein, a component of mTORC2 pathway, results in a smaller neuronal phenotype. In this study, we explored mTOR pathway molecules in MeCP2 A140V mutant mice and found a significant decrease in the levels of Rictor protein in brain homogenates of male MeCP2 A140V animals. We also compared gene expression profiles (male A140V to WT) in layer 4/5 cortical pyramidal neurons of 2-week old male mice using mouse whole genome expression arrays. Array analysis indicated that there is an alteration of IGF-1 pathway genes which are implicated in the pathogenesis of Rett syndrome. These results demonstrate that the major cell size regulator pathways involving IGF-1 and mTORC2 are significantly down regulated in MeCP2 A140V mice. Together, these findings suggest that MeCP2 A140V mutation modulate mTORC2 and IGF-1 pathways resulting in smaller neurons, which may contribute to neurologic manifestations seen in Rett syndrome.

Disclosures: S. Rangasamy: None. **S. Olfers:** None. **V. Narayanan:** None.

Poster

532. **Rett's, Fragile X, and Angelman's Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 532.26/S6

Topic: C.07. Developmental Disorders

Title: Functional analysis of MeCP2, the Rett syndrome responsible factor, in neural stem cells

Authors: *H. NAKASHIMA, K. TSUJIMURA, I. KOICHIRO, K. NAKASHIMA;
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Abstract: Rett syndrome (RTT) is a severe neurodevelopmental disorder affecting almost exclusively females. RTT is caused by sporadic mutations in the X-linked transcriptional regulator methyl-CpG binding protein 2 (*MECP2*) gene. Previous study has shown that MeCP2 is involved in neuronal maturation rather than fate specification of neural stem cells (NSCs). However, a recent study indicated that astrocytic differentiation of MeCP2 deficient mouse ES cell-derived NSCs was dramatically accelerated. In addition, we have previously shown that overexpression of MeCP2 in mouse NSCs promoted neuronal differentiation while it inhibited astrocytic differentiation. Thus, the functional roles of MeCP2 in NSCs remain ambiguous. Using both gain-of-function and loss-of-function approaches, we here examined whether MeCP2 is indeed implicated in the process of NSCs fate determination. We first found that MeCP2-overexperssion and -knockdown had no significant effect on either proliferation or cell death of NSCs. Second, we observed that the reduction of MeCP2 expression with shRNA promoted astrocytic differentiation at the expense of neuronal differentiation of NSCs. Consistent with the results of the knockdown experiments as above, enhanced astrocytic differentiation of NSCs derived from MeCP2 knockout (KO) fetal brain was detected. We confirmed that protein levels of astrocyte specific markers such as glial fibrillary acidic protein (GFAP) and aldehyde dehydrogenase 1 family, member L1 were increased in MeCP2-KO cortex and spinal cord compare to those in wild-type. Intriguingly, it has also been reported that protein levels of GFAP is upregulated in RTT patients.
Taken together, our findings suggest that MeCP2 plays important roles in fate determination of NSCs *in vitro* and *in vivo*.

Disclosures: H. Nakashima: None. **K. Tsujimura:** None. **I. Koichiro:** None. **K. Nakashima:** None.**Poster**

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.01/S7

Topic: C.07. Developmental Disorders

Support: MRC Grant G0902375

Title: Stimulus reconstruction using EEG reveals impaired low frequency speech envelope encoding (< 8Hz) in developmental dyslexia

Authors: *A. J. POWER, U. GOSWAMI;
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Abstract: Developmental dyslexia is a specific difficulty in acquiring reading found across languages and orthographies. Cognitively dyslexia is characterised by difficulties in identifying and manipulating speech sounds - a 'phonological deficit'. Auditory cortical oscillations have been proposed to play an important role in phonological encoding and speech perception. Phonological encoding is thought to involve temporal 'sampling' of information from the speech stream at different rates, phase-resetting ongoing oscillations so that they are aligned with similar envelope modulation rates in the input. Information from these modulation rates is then bound together for speech perception. Slow auditory cortical oscillations (< 8Hz) entrain to the low frequency envelope information in speech, important for syllabic parsing and encoding prosodic structure. It has been hypothesised that the encoding of the slower frequency information is impaired in developmental dyslexia, leading to the observed phonological deficit (Goswami, 2011). An increasingly popular method for assessing the extent of encoding of auditory stimuli is stimulus reconstruction (Mesgarani et al., 2009). This method determines what acoustic information can be reconstructed from recorded neural activity. Comparing this neural reconstruction to the actual stimulus gives a measure of encoding accuracy. Here we employ this method to assess low frequency speech envelope encoding in children with developmental dyslexia (DY) as well as typically developing age matched control subjects (CA) and reading age matched (younger) controls (RA). Participants were presented with 8-channel noise vocoded speech stimuli. The sentences were four words long. Subjects were asked to repeat the sentences aloud and were scored on accuracy of word report. The stimuli were semantically unpredictable but grammatically correct sentences (e.g. Arcs blew their cough). This was done to eliminate potential compensatory contextual cues within the sentences. We found that DYs and RAs performed significantly worse than CAs in the word report task. EEG was also recorded while performing the task and the stimulus reconstruction procedure was applied to the data. We found the quality of low frequency envelope encoding to be significantly worse in DYs than CAs. RAs did not differ from either group. Furthermore, the quality of low frequency envelope encoding was significantly related to behavioural measures of phonological awareness as well as to performance on the word report task. These results provide support for the temporal sampling framework theory of developmental dyslexia (Goswami, 2011).

Disclosures: A.J. Power: None. **U. Goswami:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.02/S8

Topic: C.07. Developmental Disorders

Title: Development of white matter in children with developmental dyslexia

Authors: *I. KRAFT, M. A. SKEIDE, J. BRAUER, A. ANWANDER, A. D. FRIEDERICI; Neuropsychology, Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: During their first years at school children learn how to read and to write. These two skills are crucial not only for educational success but also for an active participation in social life. However, one out of five school children suffer from dyslexia, a development disorder which is characterized by impairments in the domain of reading and writing, despite age-typical development of other cognitive abilities. A deficit in phonological awareness is discussed as one of the impairments underlying dyslexia.

The goal of our study was to investigate the relation between white matter integrity and phonological awareness as well as reading skills in 10-year old German children. We acquired diffusion tensor imaging (DTI) data from 41 children (24 males, 17 females) in order to measure individual fractional anisotropy (FA) values within the entire white matter skeleton. In addition, we used standardized psychometric tests to assess the basic reading skills and the phonological awareness of each participant.

A regression analysis revealed that the both test scores are significantly associated with FA values of the inferior longitudinal fasciculus (ILF), which is in line with previous findings from English suggesting an involvement of the ILF in reading (Rimrodt et al., 2010; Yeatman et al., 2012). Moreover, the psychometric measures are significantly related to FA values in the anterior corona radiate (ACR) suggesting an involvement of the ACR in phonological manipulation as it has been associated with working memory tasks (Nagy et al., 2004; Niogi & McCandliss, 2006; Olesen et al., 2003).

Taken together, our results confirm that white matter fractional anisotropy can serve as a neurobiological marker for a developmental dyslexia also for German, and thus possibly across languages. In particular our results suggest that the ACR is not only involved in pure working memory tasks, but also in basic phonological processing, which is an important prerequisite for reading and writing. In the future, further longitudinal studies are needed to investigate the interplay of altered fiber maturation and impaired phonological awareness as well as reading skills during childhood.

Disclosures: I. Kraft: None. **M.A. Skeide:** None. **J. Brauer:** None. **A. Anwander:** None. **A.D. Friederici:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.03/S9

Topic: C.07. Developmental Disorders

Support: NSF IGERT Grant 1144399

Title: Language deficits in autism and assessment of the Cntnap2 mouse

Authors: *A. RENDALL, D. T. TRUONG, B. C. CASTELLUCCIO, I. M. EIGSTI, R. H. FITCH;
Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with core symptoms that include atypical social interactions, language impairments, and repetitive behaviors. This developmental disorder has a strong yet complicated genetic basis, with at least 100 risk genes identified so far. One of these genes, contactin associated like protein 2 (*CNTNAP2*), was first associated with Specific Language Impairment and more recently has been linked to ASD. *CNTNAP2* is located on chromosome 7 and is downregulated by forkhead box protein 2 (*FOXP2*), a gene involved with speech and language. *CNTNAP2* is responsible for encoding a cell adhesion protein that regulates signal transmission at the synapse. In addition, *Cntnap2* has been found to promote myelin formation and speed/efficacy of signal transmission. Therefore, disruption of *CNTNAP2* may impair synapse formation, resulting in disruptions in language development - - a core feature of ASD. Clinically relevant research with language impaired groups including ASD populations has found that low-level deficits in temporal auditory processing may impair language development. In contrast, hyper-acute (e.g., improved) pitch of human speech may correlate with early language delays in ASD. To better understand the behavioral and biological underlying mechanisms of ASD, a transgenic mouse model was generated with a genetic knockout (KO) of the rodent homolog of *CNTNAP2* (*Cntnap2*). Previous studies of this model investigated by Peñagarikano et al. in 2011 reported poor social interactions, behavioral perseveration, and reduced vocalizations. The current study was designed to further assess the intermediate behavioral phenotype of this mouse model, focusing on auditory processing phenotype in this mouse model, including rapid temporal auditory processing and pitch discrimination abilities. Results show that *Cntnap2* KO mice exhibit significant deficits in rapid auditory processing as well as significant strengths in pitch discrimination. These findings suggest that *CNTNAP2* may have an underlying role in the development of neural systems important to auditory temporal processing, and disruption of this function could be associated with language impairments in ASD.

Disclosures: A. Rendall: None. **D.T. Truong:** None. **B.C. Castelluccio:** None. **I.M. Eigsti:** None. **R.H. Fitch:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.04/S10

Topic: C.07. Developmental Disorders

Support: NIH grant HD057853-01A2

Title: Auditory processing and memory impairment in mice with a genetic knockout of Dcdc2, the rodent homolog of a candidate dyslexia risk gene

Authors: *D. T. TRUONG[1], A. CHE[2], A. R. RENDALL[1], C. E. SZALKOWSKI[3], J. J. LOTURCO[2], R. H. FITCH[1];
[1]Behavioral Neurosci., [2]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [3]Dept. of Biotechnical and Clin. Lab. Sci., Univ. of Buffalo Sch. of Med., Buffalo, NY

Abstract: Developmental dyslexia is one of the most commonly diagnosed neurobehavioral disorders in school aged children and is characterized by a chronic impairment of reading ability. Gene variants in the region of DCDC2, a neurally expressed candidate dyslexia risk gene, have consistently been associated with reading disability in several independent samples. Moreover, DCDC2 variants have been associated with impairments in phonological processing and working memory ability within Dyslexic populations. To better examine the behavioral and biological underpinnings of DCDC2, transgenic mouse models have been developed, and initial behavioral characterization of mice with a genetic knockout (KO) of the rodent homolog of DCDC2 (Dcdc2) has found evidence of visuo-spatial learning impairments on the Hebb Williams Maze. To our knowledge, auditory processing ability (a component of phonological processing in humans) has not yet been assessed in Dcdc2 KO mice. The current study was designed to further expand upon clinical as well as previous behavioral findings in rodents by examining auditory processing ability and both working and reference memory ability in Dcdc2 KO mice. Auditory processing ability was measured using an embedded tone task -- a paradigm previously adapted in neuroanatomical rodent models of dyslexia. Both working and reference memory ability was examined concurrently using a water version of the 4/8 spatial radial arm maze. Results from this study not only reveal an auditory processing impairment in Dcdc2 KO mice, but also a profound memory impairment in Dcdc2 KO mice in comparison to wildtype controls across both working and reference memory domains. Further analysis of strategy to solve the water maze revealed

that both Dcdc2 and wildtype controls utilized comparable spatial strategies to navigate the maze, suggesting that increased errors made by Dcdc2 KO mice were a result of a spatial memory impairment and not due to the utilization of kinesthetic (chaining) strategies to solve the maze. Overall, these data support clinical findings, and also expand upon rodent work suggesting that disruption of DCDC2 is associated with phonological and memory impairment. Furthermore, since both phonological and working memory deficits comprise a prominent component of dyslexia, the current results provide insight to the underlying behavioral etiology of reading dysfunction that may be mediated by alteration in DCDC2 function.

Disclosures: D.T. Truong: None. **A. Che:** None. **A.R. Rendall:** None. **C.E. Szalkowski:** None. **J.J. LoTurco:** None. **R.H. Fitch:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.05/S11

Topic: C.07. Developmental Disorders

Title: Speech sound processing deficits and training-induced neural plasticity in rats with dyslexia gene knockdown

Authors: *T. M. CENTANNI[1], A. B. BOOKER[2], F. CHEN[2], C. T. ENGINEER[1], A. M. SLOAN[1], K. TRULL[2], N. WASKO[2], R. L. RENNAKER[1], J. J. LOTURCO[2], M. P. KILGARD[1]; [1]Univ. of Texas At Dallas, Richardson, TX; [2]The Univ. of Connecticut, Storrs, CT

Abstract: Reduced expression of the dyslexia associated gene Kiaa0319 in rats (KIA-) causes degraded responses to phoneme stimuli as well as increased trial-by-trial variability in onset latency. We have previously shown that in utero RNAi of this gene causes increased trial-by-trial variability in auditory cortex responses to tone and speech sound stimuli. Here we report that in utero RNAi of this gene causes significant behavioral speech sound processing impairments in rats. KIA- rats needed twice as much practice on a speech discrimination task to perform at control levels. The percentage of neurons affected by RNAi is strongly correlated with speech discrimination ability. KIA- rats are able to learn difficult speech discrimination tasks, but require long training periods and benefit from focused training using truncated speech sounds. Extensive behavioral training (>10 weeks) was able to restore trial by trial neural firing variability. This amount of training also restored (to control levels) the ability of primary and posterior auditory cortices to accurately encode speech sound stimuli. These results provide the first direct evidence that in utero suppression of the dyslexia associated gene KIAA0319 can

cause behavioral phoneme processing impairments and extensive training can ameliorate both behavioral and neural firing deficits.

Disclosures: T.M. Centanni: None. **C.T. Engineer:** None. **A.M. Sloan:** None. **R.L. Rennaker:** None. **M.P. Kilgard:** None. **A.B. Booker:** None. **F. Chen:** None. **J.J. LoTurco:** None. **K. Trull:** None. **N. Wasko:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.06/S12

Topic: C.07. Developmental Disorders

Support: UPAEP 30108-1030

Title: Rehabilitation of children with visospatial dysgraphia using a pattern recognition system

Authors: *V. REYES[1], J. M. CASTRO-MANZANO[2], J. LOPEZ-MARTINEZ[3], K. CRUZ-SÁNCHEZ[5], M. E. FLORES-SOSA[4];
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Abstract: Writing requires knowledge of a phonological system, an ability to transform phonemes into graphemes, specific movements to draw letters properly and an efficient use of space to distribute, separate and join syllables. These aspects of writing are perturbed in children with visospatial dysgraphia. The symptoms include omissions and additions of letters, inability to separate words and difficulties to maintain horizontality while writing without lines. A very common error due to dysgraphia is the distortion and rotation of letters with similar form, for example, children with dysgraphia misperceive the pairs (b,d), (p,q) and (6,9). Moreover, children with this condition also present problems in calculating, reading, building or solving puzzles. Since this whole situation increases the risk of failure and school desertion, it is necessary to generate new methods to avoid children failure using highly stimulating tools in order to motivate them to succeed. That is why our project consisted of the following: we developed a computer program (videogame inspired) that children can use with a graphic pen-tablet. Children are required to write specific letters that the program, consisting of a simple pattern recognition system, identify so that it can give users a positive or negative feedback depending on their performance and using a goal-directed approach. Our preliminary results, from twenty regular students, support previous findings showing that children are highly

motivated to work with our system. Results from 15 dysgraphic children are currently being analyzed. Financial support from UPAEP 30108-1030

Disclosures: V. Reyes: None. **J.M. Castro-manzano:** None. **J. Lopez-Martinez:** None. **K. Cruz-Sánchez:** None. **M.E. Flores-Sosa:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.07/S13

Topic: C.07. Developmental Disorders

Support: NIH Grant HD057853

Title: Induced neocortical neuronal migration disorder affects cell number in the ventral cochlear nucleus

Authors: G. C. JOHNSON, W. T. ADLER, M. P. PLATT, K. A. WRIGHT, *G. D. ROSEN, A. M. GALABURDA;
Neurol., Beth Israel Deaconess Med. Ctr., BOSTON, MA

Abstract: Previous research demonstrated that individuals with developmental dyslexia have neocortical neuronal migration disorders, including microgyria. Further, developmental dyslexics also have difficulties in rapid processing of visual and auditory information. In rats, neonatal freeze injury to the cortical plate results in malformations resembling focal microgyria. In males, induced microgyria leads to changes in cell size distribution in the medial geniculate nucleus and defects in processing rapid auditory stimuli. In contrast, female littermates with identical malformations have no behavioral or anatomic consequences. In this experiment, we tested whether induced neocortical malformations in male and female rats disrupt the cellular architecture of a brainstem auditory nucleus, the ventral cochlear nucleus (VCN).
We examined male and female rats with unilateral or bilateral microgyria induced in either the parietal or temporal cortices against sham surgery. We estimated the numbers and sizes of neurons in the VCN using optical fractionator and nucleator probes, respectively. We found fewer neurons in the VCN of males and females, combined, following temporal vs. parietal microgyria. In males, there were significantly fewer cells in the VCN in both parietal and temporal microgyria groups when compared to sham littermates. By contrast, there were no significant differences among the groups in females. Further, the decrease in neuronal number among males was more pronounced in the VCN contralateral to the microgyria than the ipsilateral VCN. There were no differences in cell sizes between microgyric rats and controls for

either sex or both sexes combined. This is the first demonstration of histometric changes at the level of the brainstem associated with focal neuronal migration disorders indicating far-reaching plasticity effects. We argue that the brainstem changes could be directly responsible for the low level auditory processing defects exhibited by rats with this injury.

Disclosures: G.C. Johnson: None. **W.T. Adler:** None. **M.P. Platt:** None. **K.A. Wright:** None. **G.D. Rosen:** None. **A.M. Galaburda:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.08/S14

Topic: C.07. Developmental Disorders

Support: Wellcome Trust

Deafness Research UK

UCL Impact Award

Title: Physiological but not anatomical abnormalities in the auditory thalamus of ectopic BXSB/MpJ-Yaa mice

Authors: *J. MATTLEY[1], L. A. ANDERSON[1], J. F. LINDEN[1,2];
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Abstract: In the BXSB/MpJ-Yaa inbred mouse strain, approximately half the animals spontaneously develop nests of displaced neurons in cortical layer I, called ectopias. Neocortical ectopias have also been reported to occur in humans with developmental disorders such as dyslexia. Interestingly, although the ectopias occur outside the auditory cortex, both human studies of spontaneously occurring ectopias and studies of rats with induced cortical malformations have observed an association with anatomical abnormalities within the auditory thalamus. Previously, we have shown that ectopic BXSB/MpJ-Yaa mice have a physiological deficit in auditory thalamic processing, despite apparently normal hearing sensitivity. The aim of this current study was to determine whether the animals also have anatomical abnormalities in the auditory thalamus.
Following in vivo electrophysiological recordings, brain tissue was processed for histology. Alternate coronal brain sections were stained for cytochrome oxidase to differentiate thalamic

subdivisions, and Nissl substance to reveal cortical ectopias and thalamic cell density. Volumes of cortical ectopias and auditory thalamic subdivisions were calculated using area estimates obtained by drawing borders on images of stained sections. Cell packing densities were estimated with a pixel-based density index, calculated from the distributions of pixel intensities across different image sections for the same subdivision and animal. All ectopic animals had at least one ectopia located in the motor cortex. Anatomical analysis showed no significant differences in thalamic volume or cell packing density between ectopic and non-ectopic mice, for either the whole auditory thalamus or any individual subdivision, even though positive controls for detection of differences between subdivisions were highly significant. These results suggest that previously observed physiological deficits in the auditory thalamus of ectopic BXSB/MpJ-Yaa mice might arise outside the auditory thalamus, or from causes with no anatomical correlate.

Disclosures: J. Mattley: None. **L.A. Anderson:** None. **J.F. Linden:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.09/S15

Topic: C.07. Developmental Disorders

Support: ERC grant

Title: Differential contributions of Foxp2 to motor-skill learning

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Abstract: Disruptions of the *FOXP2* gene cause a severe developmental speech and language disorder. This has been well studied in the KE family where a heterozygous *FOXP2* mutation is dominantly inherited. Affected individuals have difficulty producing the sequences of orofacial motor movements necessary for fluent speech. This feature has been proposed to be central to the disorder, although other expressive and receptive language problems also exist. *FOXP2* encodes a transcription factor that is expressed in the cortico-striatal and cortico-cerebellar circuits required for sensorimotor integration and motor-skill learning, and imaging studies of the KE family have shown structural abnormalities in the caudate nucleus and ventral cerebellum. The FOXP2 protein is also highly conserved in other vertebrate species, with only 3 amino acid changes between humans and mice. Mice carrying the KE-family mutation (*Foxp2-R552H/+*)

have motor-skill learning deficits and lack striatal long-term depression. We also showed that they have altered *in vivo* striatal activity during the learning of a motor task. We are now deleting Foxp2 from A. selected brain regions (cortex, striatum or cerebellar Purkinje cells) and B. a defined time point (adulthood). This genetic approach is being combined with an operant motor-sequence learning task which allows us to examine the microstructure of animals' behaviour. In the first phase of training, mice must complete a sequence of 8 lever presses to obtain a food reward. After 12 days a time constraint is added and the sequence must be performed at increasingly high speeds. Surprisingly, initial data showed that the press rate of *Foxp2-R552H/+* and heterozygous knockout mice was faster than that of controls, in contrast to cerebellar mutants which had a slower press rate. A decreased press rate was also evident in striatal mutants during the high-speed phase of the task. Histograms of inter-press-intervals revealed more subtle changes and distribution differences were evident in all mutant lines relative to controls. Lever presses were also divided into bouts (sequences of presses) which evolved during training. *Foxp2-R552H/+* and heterozygous knockouts produced sequences of a shorter duration with reduced inter-sequence intervals, whereas cerebellar mutants produced sequences of a longer duration with increased inter-sequence intervals. These data suggest that Foxp2 function in distinct subcircuits may differentially affect motor-skill learning.

Disclosures: C.A. French: None. **C. Feliciano:** None. **M. Correia:** None. **V.B. Paixão:** None. **X. Jin:** None. **S.E. Fisher:** None. **R.M. Costa:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.10/S16

Topic: C.07. Developmental Disorders

Support: DOD W81XWH-12-1-0081

NIMH R34 089299

Carol Moss Spivak Foundation

Title: Altered default mode network connectivity in neurofibromatosis-1

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Abstract: OBJECTIVE: Neurofibromatosis 1 (NF1) is caused by a mutation in the neurofibromin gene, and represents one of the most common single gene causes of learning disabilities. NF1 is characterized by impairments in frontally-mediated cognitive functions, such as attention, working memory, and inhibition, as well as significant social dysfunction. However, little is known about the functional connectivity (FC) of the resting brain in NF1.
METHOD: Two separate seed-based-analyses of resting-state functional MRI data were used to investigate FC associated with the posterior cingulate cortex (PCC) and with the ventromedial prefrontal cortex (vmPFC), known hubs of the Default Mode Network (DMN), in 12 youth with NF1 and 15 typically developing controls. The groups did not differ in terms of age, gender and translational or rotational motion during the scan.
RESULTS: FC maps derived from the PCC seed region recapitulated the classic DMN in both healthy controls and individuals with NF1, with significant connectivity between the PCC, lateral parietal cortices and the vmPFC apparent in both groups. However, relative to controls, NF1 participants showed significantly weaker FC between the PCC and other DMN nodes, including the vmPFC and left frontal pole. FC maps derived from the vmPFC seed region also revealed significant within-network connectivity between the vmPFC, lateral parietal cortices and the PCC in both groups. However, relative to controls NF1 participants showed significantly reduced FC between the vmPFC and the PCC.
CONCLUSIONS: We observed reduced strength and extent of DMN activity in subjects with NF1, with typically developing controls showing more robust long range connectivity between the two main hub regions of the DMN. Given that the DMN is active both in passive resting states and in social information-processing, these findings suggest a possible neurobiological substrate for social deficits in NF1.

Disclosures: M. Schreiner: None. **K.H. Karlsgodt:** None. **N. Enrique:** None. **T. Rosser:** None. **A. Silva:** None. **C.E. Bearden:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.11/S17

Topic: C.07. Developmental Disorders

Support: Clarendon Fund Fellowship

Stammer Trust Grant

Title: Speech-related brain activity in stuttering and cluttering: Common dysfunction in the motor network

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Abstract: Stuttering and cluttering are disorders affecting the flow of speech. People who stutter (PWS) typically know what they want to say but produce speech characterized by repetitions and prolongations of speech sounds and silences. The speech of people who clutter (PWC) is disorganized or rapid, and thought to reflect a problem in both planning and execution of speech. PWS show structural and functional abnormalities in the speech and motor system relative to controls (CON). The neural correlates of cluttering are unknown, however. Here, we explored brain activity during overt picture description and sentence reading using sparse-sampling functional MRI (3T, 32 axial slices 4 mm ^{3}, TR=9s (7s delay), TE=30ms) in 17 PWS (aged 19 - 54 yrs, 4 females), 17 PWC (aged 20 -55 yrs, 4 females), and 17 CON (aged 19 - 53 yrs, 4 females). Activity in each condition relative to a silent baseline in PWS and PWC was compared to CON and thresholded at z>2.3, $p < .01$ (uncorrected). In general, overt speech production evoked overactivity in PWC relative to CON and underactvity in PWS. Specifically, in both speaking conditions, PWC had greater activity than CON in ventral premotor cortex bilaterally, pre-supplementary motor area and right superior temporal suclus, whereas PWS had less activity than CON in right pars opercularis, parietal operculum and angular gyrus. In addition, during picture description only and in comparison with CON, PWC showed greater activity in right ventral striatum, whereas PWS had greater activity in ventral premotor cortex bilaterally and pre-supplementary area; both PWC and PWS showed less activity in lateral cerebellum bilaterally. During reading only, PWC had less activity relative to CON in left pars orbitalis and hippocampus while PWS showed less activity in the middle frontal gyrus, angular gyrus and caudate nucleus bilaterally and the left pars opercularis and pars orbitalis. In summary, PWC and PWS showed the same pattern of abnormally high activity in the ventral premotor cortex bilaterally and pre-supplementary motor area accompanied by reduced activity in the lateral cerebellum during picture description. Notable differences between the groups were also seen; the dorsal striatum was underactive in PWS whereas in PWC the ventral striatum was overactive. Our results support the idea that these two fluency disorders share a common underlying pathology but that differences between them exist and reflect group differences in the specific deficits in execution and planning of speech that characterise these two disorders.

Disclosures: E.L. Connally: None. **D. Ward:** None. **C. Pliatsikas:** None. **K.E. Watkins:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.12/S18

Topic: C.07. Developmental Disorders

Support: NIH Grant F31MH078678

NIH Grant HD053862

NIH Grant HD024064

The Simons Foundation

Title: GABAergic neuron-specific gene expression profiling in models of Rett Syndrome

Authors: *H.-T. CHAO[1,2], P. YU[2], M. HEIMAN[3], S. GONG[4], N. HEINTZ[4], J. L. NEUL[2], C. ROSENMUND[5], H. Y. ZOGHBI[2], C. A. SHAW[2];
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Abstract: Rett syndrome (RTT) is caused by mutations in *MECP2* the gene encoding methyl-CpG-binding protein 2 (MeCP2), a protein implicated in chromatin remodeling. RTT is characterized by neurological regression, impaired cognitive and motor function, repetitive stereotypies, and altered social behavior. We found that cell type specific MeCP2 deficiency in GABAergic neurons recapitulates nearly all features of RTT, suggesting that MeCP2 is a critical factor in the regulation of GABAergic function and that dysfunction of GABAergic signaling is critical in RTT pathogenesis. Previous gene expression studies revealed large numbers of transcriptional changes associated with constitutive MeCP2 deficiency, however it remains unclear how these changes are related to loss of MeCP2.
To elucidate the extent to which transcriptional changes are due primarily to loss of MeCP2 or are secondary to the cellular context of MeCP2 deficiency, we utilized models of constitutive MeCP2 deficiency (*Mecp2*$^{-/y}$) and GABAergic neuron-specific MeCP2 deficiency (*Viaat-Mecp2*$^{-/y}$) that recapitulate nearly all major RTT features. We used the *vesicular inhibitory amino acid transporter* (*Viaat*) promoter to express ribosomal protein L10 fused to enhanced green fluorescent protein (*Viaat-L10FP*) in GABAergic neurons for enrichment of actively transcribed mRNA specifically from GABAergic neurons. We compared cortical GABAergic neuron specific gene expression profiles from *Mecp2*$^{-/y}$ and *Viaat-Mecp2*$^{-/y}$ models and found that the majority of transcriptional alterations are not shared between the two models, suggesting that GABAergic specific transcriptional alterations associated with loss of MeCP2 may be

predominantly influenced by the cellular context of MeCP2 deficiency. These findings will enable us to identify primary transcriptional changes associated with MeCP2 deficiency in GABAergic specific neurons, examine the role of the cellular context of MeCP2 deficiency on transcription, and could provide an avenue to further elucidate MeCP2 function in GABAergic neurons and RTT pathogenesis.

Disclosures: C.A. Shaw: None. **P. Yu:** None. **M. Heiman:** None. **S. Gong:** None. **N. Heintz:** None. **J.L. Neul:** None. **C. Rosenmund:** None. **H.Y. Zoghbi:** None. **H. Chao:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.13/T1

Topic: C.07. Developmental Disorders

Support: KAKENHI 19390291

KAKENHI 22390216

Title: Maternal immune activation impairs the maternal-fetal leukemia inhibitory factor signal relay and reduces neural stem/progenitor cell proliferation

Authors: *T. TSUKADA[1], E. SIMAMURA[2], H. SHIMADA[2], T. AKAI[3], H. IIZUKA[3], T. HATTA[2];
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Abstract: Epidemiological studies suggest that maternal infection increases the risk of schizophrenia and autism in human offspring. Further, recent studies suggest that maternal immune activation (MIA) by infection is a cause of schizophrenia and autism in rodent offspring. Interleukin-6 (IL-6) induced by MIA is a possible key mediator of the effects of MIA on fetal brain development. However, the effects of maternal IL-6 on fetal neurogenesis are unclear. We previously showed that the maternal-fetal leukemia inhibitory factor (LIF)-adrenocorticotropic hormone (ACTH)-LIF signaling relay pathway (the maternal-fetal LIF signal relay) promotes fetal neurogenesis via the placenta in rats. We hypothesized that MIA-induced IL-6 expression in dams interferes with the maternal-fetal LIF signal relay , resulting in insufficient fetal brain development. In this study, we examined an alteration in the maternal-fetal LIF signal relay and neural stem/progenitor cell proliferation affected by MIA in mice (C57BL/6J). Polyriboinosinic-polyribocytidylic acid (polyI:C), a synthetic analog of double-stranded RNA, was used to induce

MIA. Pregnant dams were intraperitoneally injected with 20 mg/kg polyI:C at 12.5 days postcoitum (dpc). Expression of *Pomc* and *SOCS3* in placenta was examined using a gene expression assay, and quantitative PCR was performed 3 h after the injection. Concentrations of ACTH and LIF in fetal serum (FS) and that of LIF in fetal cerebrospinal fluid (CSF) were measured by ELISA at 3 h, and the concentrations of ACTH in FS and LIF in fetal CSF were also measured at 24 h after the injection. At 3 h after the injection, the mRNA level of *Pomc* in the placenta and the concentration of ACTH appeared to reduce, whereas there was no difference in the concentrations of LIF in FS and LIF in fetal CSF compared with the control. At 24 h after the injection, the concentrations of ACTH in FS and LIF in fetal CSF were both reduced. At both time points, the expression of *SOCS3* was upregulated. Using the 5-ethynyl-2'-deoxyuridine, we assessed the neural stem cell/progenitor cell proliferation at 14.5 dpc. Reduction in neural stem/progenitor cell proliferation was observed. Thus, polyI:C injection at 12.5 dpc altered the mRNA level of *Pomc* in the placenta as well as the concentrations of ACTH in FS and LIF in fetal CSF and decreased neural stem/progenitor cell proliferation. These data suggest that maternal-fetal LIF signal relay is affected by MIA, which causes impairment in neural development of fetuses.

Disclosures: T. Tsukada: None. **E. Simamura:** None. **H. Shimada:** None. **T. Akai:** None. **H. Iizuka:** None. **T. Hatta:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.14/T2

Topic: C.07. Developmental Disorders

Title: Cerebrolysin recovers behavioral and physiological impairments in an environmental rat model of autism

Authors: *A. ZWIERZCHOWSKI-ZARATE[1], S. ROYCHOWDHURY[1,2], A. BANERJEE[1], I. OGOBUIRO[1], G. FLORES[3], M. ATZORI[1,4];
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Abstract: Cerebrolysin (CBL), a nootropic drug used in Europe to treat strokes and conditions with underlying cholinergic deficits. Cerebrolysin has been proposed as a treatment for

developmental conditions like schizophrenia and diseases of the autistic spectrum disorder (ASD). Prenatal injections of valproic acid (VPA) were used as an environmental model of autism. We studied behavioral and physiologic responses in the offspring of pregnant rats injected with saline or VPA at prenatal day 12.5. The offspring of each group was split in two subgroups each, one was given chronic injections of CBL (2.5 ml/kg) and the other was treated with saline for 2 weeks.
Behavioral assays consisted in: social behavior in an open field, Y maze, and elevated plus maze. Rats were treated from P45-P60 and tested on all behavioral assays for 30 days after the injections concluded. Electrophysiological test consisted in the determination of the strength of GABAA receptor-mediated electrically evoked inhibitory postsynaptic currents currents (eIPSCs). We measured the saturation current of input/output stimulus response curves as well as the mean frequency, amplitude, and kinetics of miniature IPSCs recorded in the presence of the Na-channel blocker tetrodotoxin, in a temporal cortex slice preparation, using whole-cell patch clamp from layer II/III pyramidal neurons. We also determined the eIPSC modulation by the muscarinic agonist oxotremorine and by norepinephrine.
As expected from previous studies, the VPA injected offspring displayed impairment in all the behavioral and physiological assays used in the study. Cerebrolysin treatment recovered fully or at least partly almost all the behavioral as well as physiological alteration detected in the VPA offspring, compared to the VPA offspring untreated group, measured between 2 and four weeks after the treatment. These results suggest CBL to have potential use as a pharmacological agent for the treatment of ASD. Further studies should be conducted to determine long-term efficacy and proper treatment dose for ASD patients.

Disclosures: A. Zwierzchowski-Zarate: None. **S. Roychowdhury:** None. **A. Banerjee:** None. **I. Ogobuiro:** None. **M. Atzori:** None. **G. Flores:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.15/T3

Topic: C.07. Developmental Disorders

Support: JSPS KAKENHI 23500427

JSPS KAKENHI 00333394

Title: Study of new candidate genes that may have critical role for autism and also schizophrenia

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Abstract: Numbers of studies indicate human developmental disorders such as autism and mental retardation are mainly caused by minor dysfunctions of various genes. However, little is known about genes networks that cause neural dysfunction in cognitive development during childhood. We have been trying to identify new candidates critical for human developmental disorders by screening Drosophila and mouse genes involved in neural development.
Here we focus on new candidates, Fam107A/B that may have functions to modify actin assembly. A recent work from other group indicates Fam107A is a stress (maternal separation) response gene that alters spine density in the hippocampus. Over expression of Fam107A improves cognitive behavior such as spatial memory (Schmidt et al, PNAS, 2011). These data suggest Fam107A may have a critical role for human cognitive development. The other gene Fam107B (we call it as "Hit") is a homologous gene that have a same functional domain with Fam107B but little is known about its function in the nervous system. Using neuron-like cell line, PC12 and mouse neural primary cells, we are studying functions of these genes in vitro. Neurite outgrowth study in both PC12 and primary neurons showed FAM107B inhibits neurite outgrowth by over expression. Transwell assay showed over expression of both FAM107A/B change PC12 cells migration induced by NGF. Co-localization of F-actin and FAM107A or B was observed in outer membrane ruffles after NGF induction. To study in vivo function, we also tried gene transferring of FAM107B in utero that caused cortex migration defects. These data suggest FAM107A/B have similar but independent molecular functions in neurite extension & migration.

Disclosures: K. Koizumi: None. **M. Ito:** None. **K. Nakao:** None. **H. Nakajima:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.16/T4

Topic: C.07. Developmental Disorders

Support: ANR-06-Neuro-040-03

la Fondation Motrice

Cerebral Palsy Institute

PremUP foundation

Fondation NRJ—Institut de France

Title: A new rodent model of cerebral palsy based on prenatal ischemia and abnormal experience

Authors: M. DELCOUR[1], V. S. MASSICOTTE[3], M. RUSSIER[2], M. AMIN[3], O. BAUD[4], *M. F. BARBE[5], J.-O. COQ[2];
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Abstract: Cerebral palsy (CP) corresponds to various motor, sensory and cognitive disorders related to white matter damage (i.e. periventricular leucomalacia) often occurring after perinatal hypoxic-ischemic events. To reproduce PVL in rodents, we used a prenatal ischemia (PI) that induces white and gray matter damage. The ischemic rats exhibit visual-spatial cognitive deficits and hyperactivity, as observed in patients with CP, related to lesions of entorhinal, prefrontal and cingular cortices. Only mild locomotor disorders are induced by PI, associated to signs of spasticity, along with anatomical and functional degradation in the primary somatosensory cortex (S1), while the primary motor cortex (M1) remains unchanged. Thus, PI recapitulates the main symptoms found in children born preterm. Abnormal spontaneous movements (i.e. general movements) observed in infants who develop CP later on suggest that abnormal sensorimotor experience during maturation is key in the development of this catastrophic disease. The combination of a sensorimotor restriction (SMR) and PI in animal induces fewer cognitive deficits but still hyperactivity. Such a combination leads to severe postural and motor disorders, and spasticity, associated with musculoskeletal pathologies, as observed in patients with CP. In addition to motor disorders, drastic topographical disorganization of cortical maps in S1 and M1 suggest a major dysfunction of sensorimotor loops. Thus, our rodent model reproduces white and gray matter damage as well as the major CP symptoms. This model also shows that CP not only results from perinatal cerebral damage but from interplay between such lesions and subsequent abnormal sensorimotor experience that both contributes to increase and maintain brain dysfunctions and lesion, and in fine motor disorders, through impairments of sensorimotor integrative loops. Our promising model of CP may be valuable for exploring new strategies to prevent damage and maladaptative plasticity in immature brain.

Disclosures: M. Delcour: None. **V.S. Massicotte:** None. **M.F. Barbe:** None. **M. Russier:** None. **M. Amin:** None. **O. Baud:** None. **J. Coq:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.17/T5

Topic: C.07. Developmental Disorders

Support: Japan Society for the Promotion of Science (JSPS) Postdoctoral Fellowships for Foreign Researchers

Title: Disruption of protein homeostasis by autophagy deficiency leads to aggregation of disease-associated proteins and abnormal psychiatric behaviours

Authors: *K. K. HUI[1], A. WATANABE[2], H. MATSUKAWA[3], P. NILSSON[4], T. C. SAIDO[4], S. ITOHARA[3], T. YOSHIKAWA[2], M. TANAKA[1];
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Abstract: Although next-generation sequencing methods have enabled researchers to perform genome-wide association studies (GWAS) and examine the genetic origins of psychiatric disorders such as schizophrenia and autistic spectrum disorder (ASD), no single genetic factor have been identified to be strongly linked with either disease, therefore suggesting they are complex disorders in which multiple proteins and/or signalling pathways may be involved. Given the complex nature of these psychiatric disorders, we hypothesize that misfolding and aggregation of disease-associated proteins may play a critical role in their pathogenic mechanisms as multiple proteins may be affected simultaneously. As such, we have utilized autophagy deficiency (conditional deletion of *Atg7*) as a strategy to disrupt protein homeostasis, thereby increasing the accumulation of misfolded and aggregated protein.
We have observed that autophagy deficiency in forebrain excitatory neurons led to the accumulation of p62- and ubiquitin-positive aggregates as previously shown in other constitutive and conditional *Atg7* conditional knockout (cKO) mice. Interestingly, many disease-associated proteins were contained within these aggregates and were found to be increased in the detergent-insoluble fraction. Consistent with a reduction in the functional pool of affected proteins due to aggregation, functional assays and dendritic spine density analysis revealed significant reductions in *Atg7* cKO mice. Accordingly, deficits in long-term potentiation and other electrophysiology parameters were also observed in *Atg7* cKO mice. At the whole animal level, behavioural analysis reveals disturbances in behaviours associated with autistic spectrum disorder and schizophrenia.
The current study reveals for the first time that multiple disease-associated proteins are aggregation-prone as a result of disrupted protein homeostasis, and the resulting reduction of functional proteins may underlie the neuronal dysfunction and behavioural abnormalities. Along with recent findings from other laboratories, additional studies are warranted to provide further

evidence to support the hypothesis that protein misfolding and aggregation may be involved in the pathogenesis of psychiatric disorders such as ASD and schizophrenia.

Disclosures: K.K. Hui: None. **A. Watanabe:** None. **H. Matsukawa:** None. **P. Nilsson:** None. **T.C. Saido:** None. **S. Itohara:** None. **T. Yoshikawa:** None. **M. Tanaka:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.18/T6

Topic: C.07. Developmental Disorders

Title: A mouse model for too much TV: Unraveling the mechanisms and developmental differences in the response to sensory overstimulation

Authors: J. S. B. RAMIREZ[1], D. A. CHRISTAKIS[1,3], R. D. HODGE[2,3], R. F. HEVNER[2,3], *S. RAVINDER[4], T. K. M. RAMIREZ[1], A. F. SMITH[1], M. F. BURGOS[2], J. M. RAMIREZ[2,3]; [1]Child Hlth. Behavior and Develop., [2]Ctr. For Intigrative Brain Res., Seattle Children's Res. Inst., Seattle, WA; [3]Univ. Of Washington, Seattle, WA; [4]Ctr. For Integrative Brain Res., Seattle, WA

Abstract: With the rapid rise in media use over the past decade, infants are exposed to an influx of fast-paced sensory stimuli ranging from excessive television viewing to touchscreen applications on their mobile devices. This form of sensory overstimulation can lead to detrimental behavioral as well as neuronal changes during development. We tested this hypothesis in a mouse model in which mice (P10 - neonatal or p60 - adults) were exposed to auditory stimuli taken from the cartoon network channel and pared with visual overstimulation. Mice were stimulated for six hours per day for a total of 42 days. Overstimulation during their neonatal time period had detrimental effects resulting in diminished cognition, hyperactivity and increased risk taking (Christakis et al. 2012). Here we demonstrate, that there is a critical window in development during which the overstimulation has its greatest effects. We investigated this issue by characterizing the behavior and underlying neuronal mechanisms. Our results indicate that mice had significantly different behavioral results depending on when they were overstimulated. Moreover adult neurogenesis in the dentate gyrus was reduced in mice that were overstimulated as neonates, but there were no differences in mice that were overstimulated as adults. Our ongoing electrophysiological and optogenetic characterizations may reveal that hippocampal and amygdala activity is altered following non-normative sensory overstimulation in neonates, but not adults.

Disclosures: J.S.B. Ramirez: None. **S. Ravinder:** None. **R.F. Hevner:** None. **R.D. Hodge:** None. **D.A. Christakis:** None. **T.K.M. Ramirez:** None. **A.F. Smith:** None. **M.F. Burgos:** None. **J.M. Ramirez:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.19/T7

Topic: C.07. Developmental Disorders

Support: Jahre Foundation, Oslo

Nansen Foundation, Oslo

Title: Different subcellular distributions of AMPA and NMDA receptor subunits in two rat models of cognitive dysfunctions

Authors: A. K. LEE[1], K. S. DERVOLA[1], V. JENSEN[2], B. A. ROBERG[1], M. J. NIELSEN[1], P. STRØMME[3], Ø. C. HVALBY[2], *S. WALAAS[1];
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Abstract: Many cognitive functions appear to depend on intact glutamatergic neurotransmission in hippocampal synapses, but the extent to which specific molecular changes in these synapses are related to specific functional deficits remains unclear. The spontaneously hypertensive rat (SHR) and the inattentive ADD rat (WKY-NCrI) have been proposed as animal models for subtypes of human cognitive disorders (Sagvolden et al., 2009). We have examined the subcellular distribution of the A1 and A2 subunits from the glutamate (Glu) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors as well as the N1 subunit from the N-methyl-D-aspartic acid (NMDA) receptors, together with the postsynaptic density (PSD) marker protein PSD-95 and the cytoskeleton marker β-actin, in hippocampi from these strains and from control rats (WKY-strain). Hippocampal slices were dissected and stabilized in ACSF, followed by biotinylation to label surface proteins. Subcellular fractions were prepared and analysed by immunoblotting with specific antibodies. The Glu receptor subunits showed different distribution patterns. In the WKY controls, both the A1 and A2 subunits were enriched in the extrasynaptic membrane and cytosolic fractions, with only ~10% present in the intrasynaptic PSD fraction, whereas the N1 subunit was enriched in the PSD fraction, similar to the PSD-95 protein. Specific changes were found in the SHR and ADD animals. In the SHR animals, both A1 and A2 subunits showed decreased levels restricted to the PSD fractions, whereas the N1 subunit was unchanged in all fractions. In the ADD animal, no changes were seen in the A1 subunit, whereas

A2 subunit levels were enhanced in the PSD fraction, but decreased in the extrasynaptic membranes. Finally, levels of the N1 subunit were decreased in the PSD fraction, comparable to a 50% decrease in β-actin found in the same fraction. Our data therefore indicate that the hippocampi from the SHR and ADD rat strains have important differences in their subcellular organization, both of the glutamatergic AMPA and NMDA receptor subunits and also of the interaction between the spine cytoskeleton and PSD components. Such differences may be related to the distinct cognitive phenotypes found in these animal models.

Disclosures: A.K. Lee: None. **K.S. Dervola:** None. **V. Jensen:** None. **B.A. Roberg:** None. **M.J. Nielsen:** None. **P. Strømme:** None. **Ø.C. Hvalby:** None. **S. Walaas:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.20/T8

Topic: C.07. Developmental Disorders

Support: NIH Grant R01 HD067135

Title: Behavioral impact of *In utero* exposure to valproic acid in adult female mice

Authors: R. F. MARTIN[1], B. K. KRUEGER[2], *E. M. POWELL[1];
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Abstract: Valproic acid (VPA) is a drug used primarily in the treatment of epilepsy, but is also effective in treating bipolar disorder. Pregnant mothers taking VPA have an increased risk of giving birth to children with low IQ, delays in language and abnormal social interaction, characteristics that are associated with autism. Exposure to VPA in the late first trimester seems to present the greatest risk for developmental problems.
In the mouse, a single dose of VPA at E12.5 can produce offspring with behavioral impairments similar to those seen in autism. In this study, we hypothesize that fetal exposure to VPA alters cortical neurogenesis and/or neuronal migration, subsequently leading to impaired behavior and cognition. Previous work from our lab revealed differences in anxiety between the mice treated with VPA and those treated with vehicle control in a mostly male cohort. Sex differences were apparent, so we tested a larger female cohort. We administered several behavioral tasks to adult female mice exposed to VPA in utero to measure anxiety, motor function and general behavior, and social interaction. Overall, compared to females given vehicle in utero, there was a decrease in anxiety in females exposed to VPA. We also observed increased stereotypical behavior in both male and female offspring. These results were comparable to our previous work, demonstrating

that offspring of pregnant dams treated with VPA exhibit a behavioral phenotype similar to that observed in human offspring of mothers treated with VPA.

Disclosures: R.F. Martin: None. **E.M. Powell:** None. **B.K. Krueger:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.21/T9

Topic: C.07. Developmental Disorders

Support: NIH Grant NR010798

NIH Grant GM086257

Title: The guinea pig as a translational model for lifespan behavioral development

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Abstract: Although several excellent non-mammalian models of lifespan behavioral development exist, many studies of early neural insult and developmental disabilities require a mammalian model in order to examine changes across development in placental or maternal-fetal systems. Current mammalian models of lifespan development are either cross-sectional (rodents such as rats and mice), or prohibitively expensive for teratological studies (large mammals such as sheep or non-human primates). In this study we chose the laboratory guinea pig, *Cavia porcellus*, because of the many similarities this species shares with humans during pregnancy, precocial offspring, and social development. Using innovative methods of behavioral acclimation to ultrasonography, prenatal offspring of female IAF hairless guinea pigs (Charles River, Kingston) time mated to NIH multi-colored Hartley males (Elm Hill Labs, Chelmsford, MA) were observed longitudinally with ultrasound (GE Voluson 730 Expert) at weekly intervals across a 10 week gestation. To insure that the ultrasound procedure did not cause significant stress, salivary cortisol was collected both before and immediately after each of the observations. Measures of fetal spontaneous movement and behavioral state were quantified from video recordings from the beginning of movement at week 3 through the last week before birth. During ultrasound observations, the location of each fetus was also noted, and these individual subjects were then identified during the birth process. Postnatal offspring were observed in a series of behavioral tests, including open field and social interaction. Results from prenatal quantification of Interlimb Movement Synchrony and state organization reveal guinea pig fetal development to

be strikingly similar to that previously reported for the human fetus and preterm human infant. Consequently, this longitudinal model holds great translational promise for studying the mechanisms of developmental disabilities. More specifically, because the guinea pig is a highly social mammal, with a wide range of socially oriented vocalizations, this model may also have utility for studying the trajectories of developmental disabilities with social-emotional and/or language deficits, such as autism.

Disclosures: G.A. Kleven: None. **D. Lucas:** None. **J.S. Brewer:** None.

Poster

533. Dyslexia, Speech, and Motor Developmental Disorders

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.22/T10

Topic: C.07. Developmental Disorders

Support: NSERC of Canada

Title: Adolescent olanzapine treatment alters behavior and cortical and subcortical reorganization in adult rats

Authors: *S. RAZA[1], A. MUHAMMAD[1], R. MYCHASIUK[1], D. O. FROST[2], B. KOLB[1]; [1]Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: There is an increasing widespread use of olanzapine (OLA), an atypical antipsychotic drug, among children and adolescent populations to treat a variety of psychiatric disorders. While most animal studies, aimed at characterizing the functional sequelae of early OLA exposure, have focused primarily on developmental stages, there has been little examination of how this experience influences long-term outcomes. The present study examined the anatomical and behavioral sequelae following OLA treatment in adolescent rats. Adolescent male rats received OLA or a vehicle on postnatal days 28-49. In adulthood, rats were tested on the object/context mismatch paradigm of the novel object recognition task. Findings revealed behavioral changes associated with adolescent OLA exposure. That is, OLA-treated animals explored the novel object considerably less, relative to the vehicle group, thereby demonstrating a deficit in processing contextual information. In addition, these behavioral changes were

accompanied with alterations in the dendritic architecture of the medial and orbital prefrontal cortices and nucleus accumbens. Such findings demonstrate the consequent long-term anatomical and behavioral modulation of early OLA exposure in rats. This may have significant implications in assessing the enduring behavioral, neurobiological, and safety of OLA exposure during development.

Disclosures: S. Raza: None. **B. Kolb:** None. **D.O. Frost:** None. **R. Mychasiuk:** None. **A. Muhammad:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.23/T11

Topic: C.07. Developmental Disorders

Support: Tufts Neuroscience Institute Pilot Grant

Title: Folate deficiency: Elucidating the role of an epigenetic risk factor in developmental disorders in mice

Authors: *L. SCHAEVITZ[1], L. PAUL[2], J. SELHUB[2], J. E. BERGER-SWEENEY[1]; [1]Tufts Univ., Medford, MA; [2]Tufts Univ., Boston, MA

Abstract: One carbon (C1) metabolism plays a critical role in establishing and maintaining DNA methylation throughout life making it a promising candidate pathway to regulate epigenetic programming in developmental disorders including autism (ASD) and schizophrenia (SZ). Epidemiological studies provide evidence that increased risk for ASD and SZ is associated with both polymorphisms in C1 metabolic genes and nutritional deficiencies in C1 substrates, including folate, in both the mother and the affected offspring. Given the challenge of assessing nutritional status across the lifespan in humans, it is difficult to identify clinical symptoms that may be attributed to folate deficiency in utero (from the mother) as compared to folate deficiencies restricted to childhood or adulthood. Knowing critical time periods in susceptibility to folate deficiences can aid in understanding the etiology of and developing treatment strategies for specific symptoms of ASD and SZ. Previously we showed motor deficits, social withdrawal and cognitive dysfunction in post-weaning folate-deprived mice (Schaevitz et al., Dev Neurosci, 2012). In this study, we hypothesize that maternal folate deficiency will result in broader behavioral abnormalities as compared to post-weaning deprivation. C57Bl/6J female mice were fed a control (2 mg/kg) or folic acid deficient diet (0.4 mg/kg) for 6 weeks prior to and throughout pregnancy and lactation. Offspring were on the mother's diet until weaning. At

weaning, all offspring were fed a control diet and assessed as adults on a behavioral battery to examine repetitive/stereotyped, motor, social, anxiety, and cognitive function; behaviors affected in ASD and SZ. Adult offspring from folate-deficient mothers are less social and exhibit cognitive deficits, suggesting that early folate deficiency leads to long-lasting changes in brain function similar to those elicited by post-weaning folate deficiency. To elucidate potential molecular pathways through which altered folate metabolism affects behavior, the expression of a number of candidate proteins implicated in both ASD and SZ were examined in the prefrontal cortex. The expression of proteins involved in GABAergic function is significantly decreased in the adult offspring of folate-deficient mothers compared to the offspring of control mothers. We are currently investigating whether early folate deprivation leads to gene-specific changes in DNA methylation resulting in the dysregulated expression of proteins in the GABAergic pathway.

Disclosures: L. Schaevitz: None. **L. Paul:** None. **J. Selhub:** None. **J.E. Berger-Sweeney:** None.

Poster

533. **Dyslexia, Speech, and Motor Developmental Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 533.24/T12

Topic: C.07. Developmental Disorders

Title: Fluoxetine administered to juvenile monkeys upregulates the serotonin transporter and alters behavior into early adulthood

Authors: *S. SHRESTHA[1,2];
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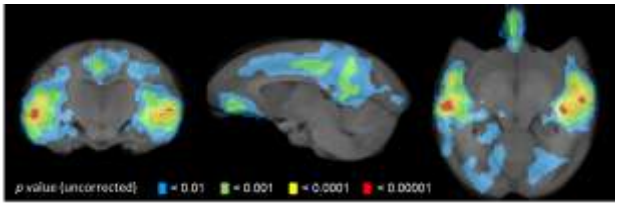
Abstract: Abstract
Objective: The use of antidepressants in children is controversial because of possible long-lasting effects on the developing brain. This study examined the long-term effects of fluoxetine administered to juvenile rhesus monkeys who, as young adults, were imaged with positron emission tomography (PET) for two serotonergic markers: serotonin transporter (SERT) and serotonin 1A (5-HT1A) receptor. An equal number of monkeys separated from their mothers at birth_an animal model of human childhood stress_were also studied.
Method: At birth, 32 male rhesus monkeys were randomly assigned to either maternal separation or normal rearing conditions. At age two, half (N = 8) of each group was randomly assigned to fluoxetine (3 mg/kg) or placebo for one year. To eliminate the confounding effects of residual

drug in the brain, monkeys were scanned at least 1.5 years after drug washout. Social interactions both during and after drug administration were also assessed.
Results: Fluoxetine persistently upregulated SERT, but not 5-HT1A receptors, in both neocortex and hippocampus. Whole-brain, voxel-wise analysis found that fluoxetine had a statistically significant effect in lateral temporal and cingulate cortices. In contrast, neither maternal separation by itself nor rearing-by-drug interaction was statistically significant for either SERT or the 5-HT1A receptor. Fluoxetine decreased dominance and increased submissive displays into adulthood in both rearing groups.
Conclusion: Fluoxetine administered to juvenile monkeys upregulates SERT and decreases dominance-like behaviors into young adulthood. This study is the first in nonhuman primates to demonstrate that an antidepressant administered during development has persistent effects into adulthood.



Disclosures: S. Shrestha: None.**Poster**

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.01/T13

Topic: C.08. Epilepsy

Support: DFG FOR 1103

Title: BUM5, a lipophilic prodrug of bumetanide but not bumetanide itself enhances the anticonvulsant effect of the GABAmimetic drug phenobarbital in epileptic mice

Authors: *C. BRANDT[1,2], K. TÖLLNER[3,2], F. TWELE[3,2], G. BRUNHOFER[4], T. ERKER[4], M. GABRIEL[4], W. LÖSCHER[3,2];
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Abstract: Rational: Recent studies demonstrate a possible role of cation-chloride-co-transporters in the pathogenesis of epilepsy. Changes in the expression pattern of the K+-Cl- co-transporter KCC2 (downregulation) and the Na+-K+-2Cl- co-transporter NKCC1 (upregulation) lead to a

GABA-shift from a hyperpolarizing to a depolarizing action caused by an accumulation of intracellular chloride. This results in a hyperexcitatory state of specific networks. In this respect the diuretic drug bumetanide has attracted growing interest. Bumetanide is an inhibitor of NKCC1 so that it is assumed that the administration of bumetanide could counteract the shift to a depolarising GABA action. Two major drawbacks of bumetanide, the diuretic potential and the low brain penetration restrict the testing of bumetanide in experimental and clinical settings.
Aim: The aim of the study is to investigate the effect of bumetanide´s prodrug BUM5 on the GABAmimetic anticonvulsant drug phenobarbital (PB) in epileptic mice. BUM5 was designed to be more lipophilic in order to penetrate into the brain and be less diuretic than bumetanide.
Methods: Drug trials were performed once a week in female NMRI mice in the maximal electroshock threshold test (MEST) beginning six weeks after a pilocarpine induced status epilepticus (SE). PB was tested alone or in combination with bumetanide or BUM5 at a dosage of 10 mg/kg i.p. with a pretreatment time of 30 min. Bumetanide (10 mg/kg i.v.) or BUM5 (13 mg/kg or 1.3 mg/kg i.v., equimolar to bumetanide) were administered 30 min before PB injection. A group of control mice without SE underwent the same test schedule.
Results: PB alone increased the seizure threshold by approx. 30% in both, control and epileptic mice. Bumetanide (10 mg/kg) and BUM 5 (1 mg/kg) did not effect the anticonvulsant effect of PB. However, at 10 mg/kg, BUM5 more than doubled the anticonvulsant effect of PB in epileptic mice, whereas such an effect was not seen in nonepileptic controls.
Conclusions: The results of this study indicate that the lipophilic prodrug of bumetanide, BUM5, could be a useful tool to study the role of NKCC1 in the pathogenesis of epilepsy and also offers a treatment option superior to the treatment with bumetanide.

Disclosures: C. Brandt: None. **K. Töllner:** None. **F. Twele:** None. **G. Brunhofer:** None. **T. Erker:** None. **M. Gabriel:** None. **W. Löscher:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.02/T14

Topic: C.08. Epilepsy

Support: Extendicare Foundation

NIH Grant T32NS043124-09

Title: Bexarotene decreases hyperexcitability in two mouse models with epilepsy

Authors: *V. C. BOMBEN[1], J. K. HOLTH[1], P. E. CRAMER[2], G. E. LANDRETH[2], J. L. NOEBELS[1];
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Abstract: Epilepsy is a complex disease with diverse environmental and genetic causes, including mutations in many ion channel genes. Moreover, epilepsy is often co-morbid with other neurological diseases such as Alzheimer's disease (AD). This makes understanding the common mechanisms between these diseases an excellent way to advance both fields of study. Recently, a cancer drug and specific retinoid X receptor agonist, bexarotene, has been shown to have a beneficial impact on a mouse model of Alzheimer's disease by reducing amyloid beta levels and improving cognitive deficits. However, the effect of bexarotene on the known neuronal network hyperexcitability of Alzheimer's mouse models has not previously been investigated, and could shed light on intermediary pathogenic mechanisms. We examined the effects of bexarotene on spontaneous cortical activity patterns in the J20 human APP mouse model of AD by EEG monitoring in awake, behaving mice. We observed that continuous oral treatment with bexarotene reduced the interictal EEG spike rate in the J20 mouse model aged 6 to 12 months. In order to further investigate this hyperexcitability reduction, we next studied a well described mouse model of epilepsy, the knockout of the voltage gated potassium channel Kv1.1. Although the Kv1.1 model does not express amyloid beta plaque pathology as seen in the J20 model, we observed by video EEG that hyperexcitabiliy, as measured by interictal spike rate, decreased over a similar course of bexarotene treatment. The effects of bexarotene progressed over several days, and were reversible. When acute hippocampal slices were bathed in 7.5mM KCl to elicit synchronous spikes within the CA3 neuronal layer, application of 10 [[Unsupported Character - Symbol Font ]]M bexarotene elicited a small but significant decrease in the spontaneous spike rate. Taken together, the in vivo and ex vivo decreases in hyperexcitability in models with and without amyloid beta plaque pathology raises important implications for understanding the mechanisms and benefits of bexarotene as a cognitive enhancer. (Research supported by NIH T32NS043124-09 and the Extendicare Foundation).

Disclosures: V.C. Bomben: None. **P.E. Cramer:** None. **G.E. Landreth:** None. **J.L. Noebels:** None. **J.K. Holth:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.03/T15

Topic: C.08. Epilepsy

Support: GW Pharmaceuticals

Title: The phytocannabinoid cannabidivarin demonstrates notable antiepileptic properties and is a genuine candidate for the treatment of temporal lobe epilepsy

Authors: *I. A. A. PÉRÈS[1,2], R. HADID[3,2], N. AMADA[3], C. L. HILL[3], A. ALHUSAINI[3], A. J. HILL[3], C. M. WILLIAMS[2], B. J. WHALLEY[3];
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Abstract: Epilepsy is a chronic and debilitating neurological condition characterised by recurrent seizures; 50 million people worldwide are affected. Temporal lobe epilepsy (TLE) is the most common epilepsy and one of the most refractory to existing medicines; ≥30% of patients are unresponsive to antiepileptic drugs (AEDs) while 20% have limited seizure control. Currently available AEDs have significant side-effects, adversely affecting alertness, motor control and cognition, making more efficacious and better tolerated AEDs an urgent requirement. Growing evidence from pre-clinical research on marijuana-derived compounds, and self-medication by epilepsy patients with marijuana, support the study of phytocannabinoids as new AEDs.
Here, the antiepileptic and tolerability profiles of a non-psychoactive phytocannabinoid, cannabidivarin (CBDV) were assessed. The lithium pilocarpine model of TLE was used to induce spontaneous recurrent seizures in male Wistar rats. Animals were treated with 200 mg/kg/day CBDV for 12 weeks and effects on seizure frequency and severity were behaviourally assessed daily for 3 weeks. CBDV significantly reduced both severe seizure incidence and seizure index (seizure severity x seizure frequency). Tolerability was assessed using static beam, grip strength and inclined screen tasks, where CBDV produced no motor function deficits or neurotoxicity. CBDV also attenuated motor function deficits observed in epileptic animals, further supporting its antiepileptic effects. Finally, electrocorticography (ECoG) recordings were used to assess the effects of acute CBDV administration (200 mg/kg) on baseline (non-seizure) neuronal activity of epileptic and non-epileptic animals. CBDV exerted few effects upon the power spectra of signals recorded from hippocampus and parietal cortex, further supporting CBDV's tolerability. In conclusion, CBDV has significant antiepileptic properties and is extremely well tolerated, supporting CBDV as a genuine candidate for the treatment of human TLE.
This work was funded by GW Pharmaceuticals and Otsuka Pharmaceutical.

Disclosures: I.A.A. Pérès: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GW Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-

kind support); GW Pharmaceuticals. **R. Hadid:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GW Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals. **N. Amada:** A. Employment/Salary (full or part-time):; Otsuka Pharmaceutical. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GW Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals. **C.L. Hill:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GW Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals. **A. Alhusaini:** None. **A.J. Hill:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GW Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals. **C.M. Williams:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GW Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals. **B.J. Whalley:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GW Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.04/T16

Topic: C.08. Epilepsy

Support: DoD W81XWH-11-1-0356

DoD W81XWH-11-1-0357

NIH RIMI P20MD001091

NIH 1R25GMO83755

LSAMP -University of Texas System

Title: Inhibitory action of levetiracetam on CA1 population spikes and dentate gyrus excitatory transmission in pilocarpine-treated chronic epileptic rats

Authors: *E. G. SANABRIA[1], L. PACHECO[1], J. ZAVALETA[1], F. SHRIVER[1], L. M. RAMBO[2], C. UPRETI[3], P. K. STANTON[3];
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Abstract: The presynaptic target for Levetiracetam (LEV) has been identified as synaptic vesicle SV2A proteins in presynaptic terminals; however, the mechanisms of LEV's antiepileptic action remain unclear. Previous studies have shown a reduction of SV2A expression in both animal models and human suffering mesial temporal lobe epilepsy (MTLE). However, in vivo treatment with LEV appears to be still effective in those conditions in ameliorating seizures. In this study, we evaluated the in vitro effects of LEV on excitability and excitatory synaptic transmission in the pilocarpine model of mesial temporal lobe epilepsy (MTLE). In this study, we investigated the action of LEV on (a) population spikes recorded in CA1 area and (b) excitatory synaptic transmission onto dentate gyrus of control versus chronically epileptic rats obtained by the pilocarpine model of MTLE. For this purpose, we used extracellular potential recordings in acutely dissociated slices. Slices were pre-incubated in 300 microM of LEV for 3 hours prior recordings. LEV was also applied in the bath during recording sections. Field excitatory postsynaptic potentials (fEPSP) were evoked by different paradigms of repetitive stimuli of perforant path (e.g. 10@20Hz). Pre-incubation with LEV induced a 20% and 10% reduction in amplitude of CA1 population spikes in slices from control and epileptic rats respectively relative to non-treated slices. LEV induced a 37.2% and 49% significant reduction in the amplitude of the summated fEPSPs in a 20Hz train evoked by perforant path stimulations in both control and epileptic groups respectively ($df=9$, $p< 0.0001$ by paired T-test) compare to baseline. Significant changes were also detected in the first four fEPSP responses in the train with a non-significant reduction of remaining 6 fEPSPs (ANOVA repetitive Test, $p<0.01$ for both groups followed by pairwise Tukey post-hoc test). These results indicate that LEV is effective in reducing in vitro excitability and excitatory synaptic transmission in both control and epileptic groups (despite possible changes in SV2A expression). Further studies are in progress to determine presynaptic mechanisms involved in this inhibitory effect.

Disclosures: E.G. Sanabria: None. **L. Pacheco:** None. **J. Zavaleta:** None. **F. Shriver:** None. **L.M. Rambo:** None. **C. Upreti:** None. **P.K. Stanton:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.05/T17

Topic: C.08. Epilepsy

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES

Title: Fish oil provides protection against the oxidative stress in the animal model of epilepsy induced by pilocarpine

Authors: *M. B. NEJM[1,1], A. A. HAIDAR[1], A. E. HIRATA[1], R. M. CYSNEIROS[2], E. A. CAVALHEIRO[1], F. A. SCORZA[1];
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Abstract: Temporal lobe epilepsy (TLE) is the most common form of epilepsy in humans and often resistant to pharmacological treatment. The pilocarpine model has been widely used due to similarity with the TLE in humans. Evidence points to the fact that neuronal lesions seen in epileptic seizures may be the result of an overproduction of free radicals (oxidative stress). Such evidence includes a positive correlation between the time of free radicals formation and the development of seizures, the protective effect that treatment with some antioxidants showed and also revealed findings such as increased lipid peroxidation. Oxidative stress is characterized by an imbalance between the antioxidant defenses and the oxidizing agents (free radicals), which can lead to tissue injury. The n-3 PUFAs are important for the development and maintenance of central nervous system functions. Our laboratory data demonstrated that chronic treatment with fish oil, immediately after status epilepticus (SE) exhibited neuroprotective and neuroplastic effects. The main propose of this research was to evaluate if n-3 PUFAS can also exhibits a protective effect against oxidative stress. Animals were subjected to the animal model of TLE by pilocarpine administration (350 mg/kg, i.p) and the controls received saline. After 3h of SE the animals were randomly divided into the following groups: animals with epilepsy treated daily with vehicle (PV) or with 85mg/kg of fish oil (PW), control animals treated daily with vehicle (CV) or with 85mg/kg of fish oil (CW). After 90 days, the enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and glutatione peroxidase (GPx) were investigate in serum and hippocampus and the expression of the SOD, CAT and NAD(P)H oxidase subunits p47 and gp91

were analyzed by western blot in hippocampus. The enzymatic activity of CAT in hippocampus, SOD and GPx in serum significantly increased in PW group comparatively to other groups, with no changes in all protein expression analysed. This finding suggests that supplementation with fish oil can provide protection against oxidative stress by increasing the activity of antioxidant enzymes.

Disclosures: M.B. Nejm: None. **A.A. Haidar:** None. **A.E. Hirata:** None. **R.M. Cysneiros:** None. **E.A. Cavalheiro:** None. **F.A. Scorza:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.06/T18

Topic: C.08. Epilepsy

Support: Grant No. 1001/PSKBP/812019

Title: Anticonvulsant effects of α-terpineol isolated from myristica fragrans on epileptic rat models and its inhibitory activity on GABAA receptor modulators in xenopus oocytes

Authors: *J. M. ABDULLAH, Prof[1], M. R. ISLAM[1,3], C. H. TARMIZI[1], H. OSMAN[2]; [1]Dept of Neurosciences, Universiti Sains Malaysia, Kelantan, Malaysia; [2]Sch. of Chem. Sci., Universiti Sains Malaysia, Minden, Malaysia; [3]Anat. and Histology, Bangladesh Agr. Univ., Mymensingh-2202, Bangladesh

Abstract: Medicinal plants continue to play a valuable role in the drug discovery process. Plant extracts can be an important source for the development of better and safer drugs for the treatment of epilepsy. Several plants that were reputed to possess antiepileptic properties in different folklore cultures have been found to exhibit anticonvulsant activities in different animal models. The aim of the present study was to investigate the antiepileptic activity of α-terpineol that isolated from Myristica fragrans Hountt. The nutmeg oil was extracted from kernels of Myristica fragrans by steam distillation and was analysed by gas chromatography-mass spectrometer (GC-MS) method. We have isolated 16 components from extracted nutmeg oil and the α-terpineol was the major components (28.05%) of nutmeg oil. We used three different doses of α-terpineol (10, 20 and 50 mg/kg; intraperitoneally) on epileptic rat models. The α-terpineol significantly reduced the seizure episodes and spikes in absence seizures model of Genetic Absence Epilepsy Rats from Strasbourg (GAERS) rats and kainic acid induced epileptic Sprague Dawley rat model by using electroencephalography (EEG) records (The EEG activity was acquired by Data Sciences International radio-telemetry system and analyzed off-line using

Neuro-Score software (St. Paul, MN, USA)). It showed a rapid onset (within 2 min) and relatively long duration of anticonvulsant effects on animal models. The dose-dependent anticonvulsant effects were comparable to the known antiepileptic drug of diazepam. We have also defined the inhibitory effects of α-terpineol at different concentration in chloride currents (IGABA) stimulation through gamma-amino butyric acid type A (GABAA) receptors comprising of α1β2γ2s subtype activity making use of a two-microelectrode voltage clamp assay on Xenopus laevis oocytes. The α-terpineol was found to be induced an enhancement of IGABA modulation (EC5-10) by 229.6 ± 23.8% and 326.3 ± 43.8%, respectively at 100 μM and 500 μM. Thus the dose dependent activity of α-terpineol showed statistical significance potentiation of IGABA in comparison to the control group. Our present findings thereby provides a scientific evidence for the traditional use of nutmeg oil for the treatment of epilepsy, and suggest that the α-terpineol may deserve to be a potential candidate of antiepileptic compounds.

Disclosures: J.M. Abdullah: None. **M.R. Islam:** None. **C.H. Tarmizi:** None. **H. Osman:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.07/U1

Topic: C.08. Epilepsy

Support: GlaxoSmithKline: GW58292-11653

Title: Determining efficacy of retigabine on acute limbic seizure threshold in adult rats

Authors: *L. K. FRIEDMAN, J. P. WONGVRAVIT, A. M. SLOMKO, S. HU, W. WAN, S. ALI, Z. NASSEER;
Cell Biol. & Anat., New York Med. Col., Valhalla, NY

Abstract: Retigabine (manufactured by GlaxoSmithKline), is the first neuronal potassium channel opener indicated for the adjunctive treatment of focal (partial) seizures in adult patients. However; little is known about the efficacy of retigabine in experimental models of partial limbic seizures. The oral LD50 for rats was previously reported at 980mg/kg. We tested systemic doses of retigabine (1, 2, 5, 10, 100, 300 mg/kg) on kainate (KA)-induced status epilepticus (SE) in adult rats 30 min prior to (and 2 hrs following) injection of KA (16 mg.kg, i.p.). Doses ≥10 mg/kg produced deep, rapid sedation and was discontinued. A single injection of 2 or 5 mg/kg was insufficient to prevent the induction of KA SE; however there was significant reduction or absence of stage5-6 seizure behavior symptoms such as pronounced drooling and forelimb

clonus in 50% of the animals. Two prior treatments (24 hrs and 30 min prior to KA injection) increased seizure behavior latency and attenuated spiking in the electroencephalogram (EEG). At 2 hrs after KA injection, during status epilepticus, 2 mg/kg of retigabine quickly calmed seizure behavior and reduced epileptiform discharges, whereas, 5 mg/kg increased EEG spiking and synchronization of burst activity. Although histological analysis revealed that animals scored with stage5-6 seizures exhibited typical hippocampal injury regardless of treatment, GluR1 subunit protein was selectively suppressed within the granule cell layer (GCL) and the mossy fiber synapse in animals treated with retigabine. Animals treated with retigabine that were resistant to KA-SE did not exhibit significant injury only indicating that retigabine was neuroprotective if seizures were prevented. Thus, it appears that lower doses of retigabine had greater efficacy producing a potent anticonvulsant effect possibly due to reduced excitatory transmission via AMPA receptors; whereas higher doses produced ataxia and proconvulsant effects in this model of epilepsy indicating that the dosage used in humans must be carefully scrutinized to produce the adequate clinical response.

Disclosures: L.K. Friedman: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; GalxoSmithKline. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GalxoSmithKline. **J.P. Wongvravit:** None. **A.M. Slomko:** None. **S. Hu:** None. **W. Wan:** None. **S. Ali:** None. **Z. Nasseer:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.08/U2

Topic: C.08. Epilepsy

Support: NS72258

NS77908

Title: An *in vitro* screen for antiepileptogenic compounds utilizing organotypic hippocampal slice cultures

Authors: *Y. SAPONJIAN[1], Y. BERDICHEVSKY[2], W. SWIERCZ[1], K. STALEY[1]; [1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Electrical & Computer Engin., Lehigh Univ., Bethlehem, PA

Abstract: The accelerated course of epileptogenesis in the *in vitro* organotypic hippocampal slice culture model of post-traumatic epilepsy (Dyhrfjeld-Johnsen et al. *J Clin Neurophysiol* 2010) was utilized to conduct a moderate-throughput screen of an array of drugs to study their antiepileptic and neuroprotective effects and ultimately find antiepileptogenic compounds.
Organotypic hippocampal slices cultures were generated from postnatal day 6 or 7 Sprague-Dawley rats and were maintained in poly-D-lysine coated 6-well tissue culture plates on a rocking platform in a humidified chamber at 37^{o}C and 5% CO_2. The culture medium consisted of Neurobasal-A, B27, 0.5 mM GlutaMAX, and 30 μg/ml Gentamicin and was changed every 3-4 days. Drugs, obtained primarily from the NINDS custom Compound Collection, were dissolved in DMSO (final concentration 0.1%) and added to the media starting on DIV 3. All experiments included DMSO control slice cultures derived from the same animal.
The lactate concentration in spent culture medium was strongly correlated with electrographic seizure activity and used in subsequent experiments as an assay for seizure activity. Lactate dehydrogenase concentration in spent culture medium was correlated with propidium iodide assays for cell death and used in subsequent experiments as an assay for the rate of cell death (Berdichevsky et al. *J Neurosci* 2013). Lactate and LDH concentrations were plotted as cumulative values over 28 days *in vitro* for chronic-application experiments.
With these technologies, one researcher was able to screen 9 conditions per week with 3 replications per condition. We investigated culture conditions, culture media components, and the anticonvulsant, antiepileptogenic, and neuroprotective effects of over 150 drugs. Drugs were typically tested at 3 concentrations: the most likely effective concentration as well as 1 log above and below this concentration. Positive screens were repeated to confirm the findings. Drugs exhibiting significant anticonvulsant activity were further analyzed in wash-out experiments to differentiate anticonvulsant from antiepileptogenic effects.
Several drugs exhibited dose-dependent anticonvulsive or proconvulsive effects, as well as neuroprotective or neurotoxic effects. The timing of drug application was an important determinant of the action of several drugs. Anticonvulsant and neuroprotective effects were strongly correlated, presumably due to reductions in ictal cell death. This technology comprises a promising tool for the rapid investigation of drug efficacy in chronic epilepsy.

Disclosures: Y. Saponjian: None. **Y. Berdichevsky:** None. **W. Swiercz:** None. **K. Staley:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.09/U3

Topic: C.08. Epilepsy

Support: CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Contract # HHSN271201100029C

Title: Ezogabine protects against status epilepticus-induced neurodegeneration and cognitive decline

Authors: *A. B. ALEX[1], S. D. DRAPER[1], K. JOHNSON[1], J. L. FITTS[1], H. S. WHITE[1,2]; [1]Anticonvulsant Drug Develop. Program, Salt Lake City, UT; [2]Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT

Abstract: Status epilepticus (SE) when left untreated leads to long-term consequences that include hippocampal sclerosis, behavioral and cognitive deficits. The cell loss and cognitive decline associated with pilocarpine-induced status epilepticus (SE) is consistent with the sclerosis observed in human temporal lobe epilepsy. Effective therapeutic intervention and suppression of seizures is essential to prevent seizure-induced neurodegeneration and cognitive decline. An ideal anti-seizure drug should not only be able to block the seizures, but also prevent the seizure-induced long-term functional consequences. The present study was undertaken to evaluate the ability of the newly approved anti-seizure drug ezogabine (EZG), to prevent neurodegeneration and subsequent cognitive decline. Systemic administration of pilocarpine (50 mg/kg; i.p.) induces prolonged seizures that last for several hours. Administration of EZG (60 mg/kg) at 30 minutes after the first stage 3 seizure halted the generalized behavioral seizures. Two weeks after SE, the rats were tested for spatial learning and memory in Morris water maze, where the task is to find a hidden escape platform. The rat's escape latency, distance traveled and swim speed were recorded. Pilocarpine alone-treated rats showed significantly higher escape latency and traveled greater distance before finding the platform. The rats treated with EZG, performed better than pilocarpine-treated rats. In addition, administration of EZG prevented the hippocampal cell death in DG, CA3 and CA1 cell layers in a majority of the animals. Results from these studies suggest that EZG, a new anti-seizure drug with a unique mechanism of action; i.e., potassium channel activator, has the potential to be a useful drug in attenuating seizure-induced cognitive decline associated with hippocampal sclerosis.

Disclosures: A.B. Alex: None. **S.D. Draper:** None. **K. Johnson:** None. **J.L. Fitts:** None. **H.S. White:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Contract # HHSN271201100029C..

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.10/U4

Topic: C.08. Epilepsy

Support: Funded by GW Pharmaceuticals plc

Title: Cannabidiol and cannabidivarin in a non-psychoactive, well defined marijuana extract exert linearly additive anticonvulsant effects against generalised seizures

Authors: *T. D. HILL[1], M. DUNCAN[3], C. M. WILLIAMS[2], A. J. HILL[1,2], B. J. WHALLEY[1]; [1]Reading Sch. of Pharm., [2]Sch. of Psychology and Clin. Language Sci., Univ. of Reading, Reading, United Kingdom; [3]GW Pharmaceuticals plc, Salisbury, United Kingdom

Abstract: Epilepsy is a chronic neurological disorder characterised by recurrent seizures. Despite advances in pharmacological treatment, ~30% of people with epilepsy have poorly controlled seizures which increase mortality and the presentation of co-morbidities. Marijuana has historically been used to treat a wide variety of diseases including epilepsy but the psychoactivity of Δ9-THC prevents widespread acceptance of medical marijuana. Cannabidivarin (CBDV) and cannabidiol (CBD) are non-psychoactive phytocannabinoids (pCBs) present in marijuana that exert significant anticonvulsant effects in acute, in vivo models of seizure.

Here, we investigated the effects of a marijuana-derived botanical drug substance (BDS) principally containing CBDV and CBD and with Δ9-THC removed in the rat pentylenetetrazole (PTZ) and DBA/2 mouse audiogenic models of acute, generalised seizure. Furthermore, an isobolographic study design was employed to determine whether the anticonvulsant effects of CBDV and CBD interacted in any way.

Animals were group housed in a 12/12 hour light/dark cycle (21°C), with food and water provided ad libitum. Animals received i.p. BDS/pCBs one hour prior to convulsant stimulus. The seizure behaviour of male Wistar rats (24-28 days old) was assessed for 30 minutes following i.p. administration of PTZ (85 mg/kg). Male DBA/2 mice (21-28 days old) were placed in a bell jar, where a bell was sounded (110-120 dB) for ≤60 seconds to induce seizures.

In the PTZ model, the BDS significantly reduced seizure severity from a seizure that was fatal in 46% of cases to a severity that caused no mortality (175 mg/kg; n=15); an effect comparable to that exerted by matched levels of co-administered isolated CBDV and CBD. In the audiogenic model, the BDS significantly reduced the incidence of wild running and clonic convulsions (≥50 mg/kg; n=10). Using isobolographic experimental design and analysis, CBDV and CBD were found to interact in a linearly additive manner to suppress audiogenic seizures.

In conclusion, a BDS containing CBDV and CBD is strongly anticonvulsant and suppresses generalised seizures with similar potency to pure CBDV and CBD when co-administered; actions independent of the presence of Δ9-THC. Furthermore CBD and CBDV's anticonvulsant actions are linearly additive when administered in combination. These results strongly support wider clinical investigation of these non-psychoactive cannabinoids in epilepsy.

Disclosures: T.D. Hill: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Drugs supplied by GW Pharmaceuticals plc. **M. Duncan:** A. Employment/Salary (full or part-time):; GW Pharmaceuticals plc. **C.M. Williams:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Grant from GW Pharmaceuticals plc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Drugs supplied by GW Pharmaceuticals plc. **A.J. Hill:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Drugs supplied by GW Pharmaceuticals plc. **B.J. Whalley:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Grant from GW Pharmaceuticals plc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Drugs supplied by GW Pharmaceuticals plc.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.11/U5

Topic: C.08. Epilepsy

Support: CONACyT grant 98386

CONACyT scholarship 226906

Title: Anticonvulsant effect of phenytoin transported by magnetic nanoparticles in an animal model of P-glycoprotein brain overexpression

Authors: *A. ROSILLO-DE LA TORRE[1], L. ZURITA-OLVERA[2], J. LUNA-BARCENAS[2], S. OROZCO-SUAREZ[3], P. GARCIA[4], L. ROCHA[1];
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Abstract: Magnetic nanoparticles (MnPs) represent a potential and novel strategy to control pharmacoresistant disorders in which the brain overexpression of P-glycoprotein (P-gp) limits the achievement of effective therapeutic concentrations of drugs. The main goal of the present study was to evaluate the effects of phenytoin (PHT) loaded in the silica core of MnPs in an animal model of brain P-gp overexpression. MnPs were synthesized by coprecipitation and then covered with silica by the sol-gel method. PHT was loaded on the silica core shell by absorption with a final concentration of 62 mg/100 mg of MnPs. P-gp brain overexpression was induced in male Wistar rats by repeated seizure induction as consequence of 3-mercaptopropionic acid (3MP) (37.5 mg/kg i.p.) applied every 12 h for 11 administrations. MnPs-3MP group (n=10) received an administration of PHT loaded in MnPs (75 mg/kg, i.p.) one hour before the last 3MP injection. Results were compared with those obtained from the following groups:a) PHT-3MP group (n=9) in which the rats were manipulated as MnPs-3MP group, but injected with PHT (75 mg/kg i.p.) not loaded in MnPs; b) 3MP group (n=7) that only received repetitive 3MP administration. Latency and incidence to mioclonus, clonic and tonic-clonic seizures were assessed during 30 min immediately after 3MP injection. Animals were sacrificed 24 h after the last 3MP or saline administration, and their brain was used to evaluate the *P-gp expression by immunochemistry. During the last 3MP administration, rats from the 3MP group showed the following seizure latency and incidence: mioclonus at 4.5±2min (100%); clonus at 7.2±1.3 min (86%); tonic-clonic at 7.4±1.35 min (71%).* PHT-3MP group presented similar incidence for mioclonus (89%), clonus (89%) and tonic-clonic seizures (78%), *when compared with 3MP group. Concerning* MnPs-3MP group, animals presented similar incidence for mioclonus (70%), but reduced number of animals showed *clonus (40% $p<0.05$) and tonic-clonic events (20%, $p<0.02$). In contrast with 3MP group, PHT-3MP and MnPs-3MP groups showed similar latencies for the different type of seizures.*Immunohistochemical analysis revealed that the repeated administration of 3MP induced the overexpression of P-gp in blood brain vessels of different brain areas. The results of the present study support the idea that MnPs can be used as a therapeutic strategy to transport antiepileptic drugs to the brain of animals with P-gp overexpression. Future studies should be focused to evaluate the beneficial and undesirable effects of MnPs in experimental models of pharmacoresistant epilepsy.

Disclosures: A. Rosillo-De La Torre: None. **L. Zurita-Olvera:** None. **J. Luna-Barcenas:** None. **S. Orozco-Suarez:** None. **P. Garcia:** None. **L. Rocha:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.12/U6

Topic: C.08. Epilepsy

Support: A grant (2012K001115) from the Brain Research Center from the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Republic of Korea

Title: Liposome-encapsulated melatonin attenuates trimethyltin-induced neurotoxicity via inhibition of protein kinase Cδ proteolysis

Authors: *M.-B. WIE[1], J. LEE[2], Y. NAM[3], J. JEONG[3], J. LEE[4], E.-J. SHIN[2], H.-C. KIM[2]; [1]Col. of Vet. Med. and Inst. of Animal Med., [2]Neuropsychopharm. & Toxicology Program, Col. of Pharm., Kangwon Natl. Univ., Chunchon, Korea, Republic of; [3]Dept. of Pharmacology, Col. of Med., [4]Col. of Pharm., Chung-Ang Univ., Seoul, Korea, Republic of

Abstract: In the present study, we examined the effect of liposome-encapsulated melatonin (LM), a potent antioxidant, on the trimethyltin (TMT)-induced neurotoxicity. Male C57BL/6 mice received LM (20 mg/kg as a melatonin) subcutaneously twice a day from 7 days before TMT (2.8 mg/kg, i,p.) injection. Additional LM was injected until 2 days after TMT. Convulsive behavior was measured at 1 and 2 days after TMT injection, and then mice were sacrificed for histological observation. Treatment with LM significantly attenuated TMT-induced convulsive behavior. Consistently, TMT-induced nuclear chromatin clumping was also significantly decreased by LM in the dentate gyrus of hippocampus. Since we and others have suggested that PKCδ plays an important role on the oxidative damage in various neurotoxic conditions, we then examined whether treatment with LM modulates the expression of PKCδ and its cleaved form after TMT injection. Hippocampal expression of cleaved-PKCδ was significantly increased in the TMT-treated mice as compared with saline-treated mice, and these changes were significantly attenuated by treatment with LM. Our results suggest for the first time that inhibition of PKCδ proteolysis mediated by LM provides a potential neurotherapeutic intervention in response to TMT-induced excitotoxicity.

Disclosures: M. Wie: None. **J. Lee:** None. **Y. Nam:** None. **J. Jeong:** None. **J. Lee:** None. **E. Shin:** None. **H. Kim:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.13/U7

Topic: C.08. Epilepsy

Title: Anticonvulsant action of the cyclooxygenase inhibitor celecoxib in an *In vitro* post-traumatic epilepsy model

Authors: *K.-I. PARK, Y. SAPONJIAN, V. DZHALA, M. MAIL, K. STALEY;
Neurol., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Celecoxib, a cyclooxygenase 2 inhibitor, has been studied as a possible anticonvulsant in numerous experimental paradigms. However, its efficacy as an anticonvulsant is still controversial. We screened a large number of compounds for anticonvulsant activity using the hippocampal organotypic slice preparation as a model of post-traumatic epileptogenesis (Berdichevsky et al. *J Neurosci* 2013). Celecoxib proved to be efficacious in the screening protocol, so we tested 1) whether the efficacy of celecoxib (10μM) was evident electrophysiologically in the slice cultures, 2) whether it altered the acute seizure activity induced by low-Mg perfusion of intact hippocampal formations and 3) whether celecoxib exhibited antiepileptogenic effects. Extracellular field potential recordings confirmed the anticonvulsant efficacy celecoxib in the organotypic slice culture model of posttraumatic epilepsy: reductions of the power of epileptic activity were 62.8% ($p<0.01$) in early stages of epilepsy and 56.9%, ($p<0.01$) in later stages. Seizure frequency decreased by 81.5% ($p=0.02$) and 67.5% in early and later stage respectively. However, celecoxib was not effective against seizure activity induced acutely in normal tissue. Celecoxib applied to slice cultures for 3 weeks followed by a 4-day washout did not prevent or alter the emergence of seizure activity, indicating that celecoxib does not exhibit antiepileptic activity in this preparation. Spontaneous seizures were observed in 44% of slices in celecoxib group and 67% in control group after washout. Total duration of seizures was longer (54.8±4.6 min vs., 18.3±3.1 min, $p<0.01$) and the number of microglia was higher 282.2±25.1/mm^2 vs., 198.9±13.4/mm^2 $p=0.03$) in celecoxib group. Astrogliosis was not significantly different. In conclusion, our data provide evidence of an acute anticonvulsant effect of celecoxib in models of chronic post-traumatic epilepsy, but not in acute seizure models based on normal brain tissue. The robust anticonvulsant effects of celecoxib in this in vitro post-traumatic model underscores the potential of cyclooxygenase as a target of chronic epilepsy treatment. The modest effects of celecoxib in acute seizure models highlights the utility of the organotypic slice culture model as a complimentary screen for drugs to treat epilepsy that does not respond to currently-available agents discovered using acute seizure models.

Disclosures: K. Park: None. **Y. Saponjian:** None. **V. Dzhala:** None. **M. Mail:** None. **K. Staley:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.14/U8

Topic: C.08. Epilepsy

Support: NIH Grant 1R01CA163452

National Center for Research Resources 5P20RR024485-02

NIH/NHLBI T32-HL007260-36

Title: Synthetic Vitamin K derivatives provide protection in multiple neurological disease models

Authors: *B. JOSEY, R. COMER, E. INKS, J. RAHN, S. CHAN, J. CHOU;
Med. Univ. of South Carolina, Charleston, SC

Abstract: For the past century, vitamin K has been known to be an essential cofactor for the γ-carboxylase activity, which is involved in blood coagulation and calcium-binding protein in bone formation. Recently, vitamin K has begun to emerge as playing a critical role in brain function, although much about its physiology in the brain remains unknown. Vitamin K has two major forms: vitamin K1 and vitamin K2. Vitamin K1, phylloquinone, is synthesized in green leafy vegetables because of its role in photosynthesis. Vitamin K2, menaquinone, is the predominant and biologically relevant form in animals and has several subtypes depending on the number of side chain isoprenoid residues. One of these subtypes, MK-4, is the dominant form of vitamin K found in human tissues, and recent studies indicate that MK-4 constitutes 98% of vitamin K found in brain tissue and is highly concentrated in the midbrain and pons medulla. Most forms of vitamin K have relatively poor bioavailability, and it is hypothesized that MK-4 is likely endogenously synthesized in situ from dietary phylloquinone, via side chain cleavage, transport, and site selective prenylation to produce tissue-specific menaquinones. The capacity to convert both pylloquinone and menadione to MK-4 by cultured brain slices has been demonstrated, further implicating an important role of the nutrient in brain physiology. Results from recent studies indicate that brain levels of VK decrease with age and that decreased VK levels correlate with cognitive decline in neurodegenerative diseases, although to date very little research has gone into characterizing the extent of its impact. The cause of this decrease is still unknown, although it is likely due to some defect in the absorption of the lipophillic molecule, the transport mechanisms involved with getting it to the brain, or in the biosynthetic machinery. These data indicate that the development of small drug-like substitutes of vitamin K that are biologically stable and capable of penetrating the blood brain barrier could be potentially valuable. We have previously reported a structure-activity relationship study of vitamin K that yielded several small molecule analogs that protected neuronal cultures from various forms of cell death. Herein, we present the characterization of synthetic VK derivatives with no observable toxicity in vivo that

demonstrate improved bioavailability and brain tissue uptake. These derivatives provide protection in multiple in vitro and in vivo models of neurological disorders, including the prevention of seizures in the murine 6 Hz corneal stimulation model, indicating potential use in the treatment of therapy-resistant epilepsy.

Disclosures: B. Josey: None. **R. Comer:** None. **E. Inks:** None. **J. Rahn:** None. **S. Chan:** None. **J. Chou:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.15/U9

Topic: C.08. Epilepsy

Support: Texas A&M Emerging Technology Funds to A.K.S.

Merit Award from Department of Veterans Affairs to A.K.S.

Title: Resveratrol treatment after the onset of status epilepticus reduces neurodegeneration and inflammation in the adult hippocampus

Authors: *V. MISHRA[1,2], B. SHUAI[1,2], B. HATTIANGADY[1,2], A. K. SHETTY[1,2];
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Abstract: Status epilepticus (SE) is a life-threatening emergency medical condition, typified by prolonged seizure activity. Currently available medications for SE such as antiepileptic drugs (AEDs) suppress seizures in most patients but do not prevent seizure-induced neurodegeneration. Furthermore, AEDs fail to modify the down-stream detrimental effects of SE such as neuroinflammation and chronic epilepsy development. Thus, efficient neuroprotective and antiepileptogenic strategies are needed for preventing SE-induced morbidity. In this study, we rigorously examined the efficacy of resveratrol (RESV), a phytoalexin found in the skin of red grapes having potent antioxidant and anti-inflammatory properties, for preventing SE-induced neurodegeneration and neuroinflammation in the hippocampus. Graded intraperitoneal injections of kainic acid (3 mg Kg bw/hour for 3-4 hours) were employed to induce SE in young adult F344 rats. Groups of rats exhibiting SE received either intraperitoneal RESV (30 mg/Kg, commencing an hour after the induction of SE and continuing every hour for 3 hours on SE induction day and twice daily thereafter for 6 days) or vehicle (VEH). Behavioral seizures were terminated in both RESV and VEH treated animals at 2 hours after the induction of SE with an injection of

diazepam (5 mg/Kg). Stereological counting of neurons expressing the neuron-specific nuclear antigen (NeuN) at 7 days post-SE revealed considerable loss of neurons in the hippocampus of rats receiving VEH after SE, in comparison to the naïve control group (64% in the dentate hilus, 19% in the granule cell layer, 40-53% in CA1 and CA3 pyramidal cell layers; $p<0.01$). Contrastingly, in rats receiving RESV after SE, these populations of neurons were largely preserved. Furthermore, RESV-treated rats displayed reduced numbers of ED-1+ activated microglia than VEH-treated rats in the CA1 and CA3 subfields (33-55% reduction, $p<0.01$), implying repression of inflammation by RESV. Additionally, RESV treated rats exhibited survival of greater numbers of GABA-ergic interneurons expressing neuropeptide Y (NPY) and parvalbumin (PV) than VEH treated rats ($p<0.05$), suggesting protection of interneurons by RESV. Thus, RESV treatment after the onset of SE is efficacious for protecting principal as well as GABA-ergic neurons, and reducing neuroinflammation in the hippocampus. As loss of GABA-ergic interneurons and neuroinflammation are believed to contribute greatly towards the evolution of initial SE-induced hippocampal injury into chronic epilepsy, RESV treatment after SE may be beneficial for restraining SE-induced chronic epilepsy and other co-morbidities.

Disclosures: V. Mishra: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); MegaResveratrol supplied Resveratrol for this study for free. **B. Shuai:** None. **B. Hattiangady:** None. **A.K. Shetty:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.16/U10

Topic: C.08. Epilepsy

Support: NIH Grant 2U01NS058162

Title: Delayed post-treatment with atropine sulfate arrests generalized seizures induced by soman in immature male rats

Authors: *S. L. MILLER[1], T. H. FIGUEIREDO[1], E. M. PRAGER[1], C. P. ALMEIDA-SUHETT[1], J. P. APLAND[2], V. ARONIADOU-ANDERJASKA[1], M. F. M. BRAGA[1]; [1]Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]United States Army Med. Res. Inst. of Chem. Def., Edgewood, MD

Abstract: Nerve agents irreversibly inhibit the enzyme acetylcholinesterase (AChE) in the peripheral and central nervous system. Following nerve agent intoxication prolonged seizures result from the hyperstimulation of muscarinic acetylcholine receptors (mAChRs) within the

basolateral amygdala (BLA). Experimental models suggest children would be highly susceptible to seizures and death following nerve agent exposure. Asoxime chloride, an oxime, and atropine sulfate (ATS), a general muscarinic antagonist, are considered the standard of care for nerve agent intoxication in adults. However, it is unknown if these drugs will be an effective delayed treatment in children. Knowledge of the effects of nerve agents on immature animals is limited. We determined the median lethal dose (1 X LD50) of soman on P21 male rats to be 62 μg/kg, which is less than 2/3 of the dose for adult male rats. We tested whether immature male rats are more sensitive to soman due to reduced AChE activity in the BLA. We found a significant reduction in AChE activity in the BLA following soman induced seizures, whereas AChE activity was reduced in the prefrontal cortex, piriform cortex and hippocampus in animals without seizures. We exmained whether differences in mAChR subtype expression within the BLA may explain the sensitivity of immature rats to soman. mAChR expression in the mouse BLA was semi-quantitatively analyzed using publically available in situ hybridization data from the Allen Institute for Brain Science. A developmental reduction in M2 mAChRs-which open potassium channels-was found and may contribute to soman sensitivity. Due to incomplete expression of all mAChR subtypes, we hypothesized a low dose of ATS would counteract soman induced seizures. We administered 2.0 mg/kg ATS 20 minutes post exposure to soman (1.2 X LD50). This dose arrested generalized seizures in 100% of treated animals. These data may explain the sensitivity of immature male rats to soman and the potent efficacy of ATS against soman induced seizures.

Disclosures: S.L. Miller: None. **T.H. Figueiredo:** None. **E.M. Prager:** None. **C.P. Almeida-Suhett:** None. **J.P. Apland:** None. **V. Aroniadou-Anderjaska:** None. **M.F.M. Braga:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.17/U11

Topic: C.08. Epilepsy

Support: French Inter-ministries Fund (Fonds Unique Interministeriel)

Région Alsace

Mulhouse Area m2A

Alsace Business Angels

Regional Venture Fund SODIV

Title: Biosimulation of mesiotemporal lobe epilepsy for the search of new antiepileptic drugs and anticipation of proconvulsant risks

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Abstract: Mesiotemporal lobe epilepsy (MTLE) is the most common form of human epilepsy, it is characterized by focal seizures, typically associated with hippocampal sclerosis (Langlois et. al. 2010). Prior to surgical intervention, physicians empirically seek a drug combination to abolish seizures. Unfortunately, MTLE is often resistant to conventional treatments, and great efforts have been made to discover new antiepileptic drugs (AEDs), with very limited success. In this study, we used a biosimulation approach to bring new insights on the roles of different molecular mechanisms that control the excitatory/inhibitory balance in hippocampus. We first developed kinetic models of a large number of receptors and transporters and assessed the effects of several AEDs (CGP-40116, Picrotoxine, Muscimol, Gabazine, Tiagabine, Carbamazepine, Lamotrigine) on these elementary models. These elementary blocks were then integrated into models of glutamatergic and gabaergic synapses, which were incorporated into a model of a CA1 pyramidal neuron (Jarsky 2005). Finally, we implemented the microcircuitry that surrounds CA1 neurons to take into account feedforward inhibition to the perisomatic area through basket cells, and feedback inhibition driven by O-LM interneurons. All elementary models were developed and validated using RHENOMS (V2) modeling and simulation platform, and then integrated to the NEURON Simulation environment (Hines 1997). The model reproduced epileptiform activities (paroxysmal depolarization shift or PDS), as well as dose-dependent responses to AEDs, in good agreement with results observed in an in vitro model of epilepsy (low magnesium concentration) and In vivo (kainate mouse model). The model was then used to analyze the effects of combinations of various doses of AEDs to detect possible synergetic or asynergetic effects. The approach illustrates the value of the multiscale simulation platform for the study of epilepsy and for the discovery of novel therapeutic agents. It allows addressing fundamental as well as translational questions, and provides a strong support for drug research and discovery, especially for the development of complex therapeutic strategies or the assessment of toxicity risks.

Disclosures: A. Legendre: None. **T.W. Berger:** None. **J.C. Bouteiller:** None. **F. Pernot:** None. **R. Greget:** None. **C. Roucard:** None. **A. Depaulis:** None. **L. Fagni:** None. **A.F. Keller:** None. **N. Ambert:** None. **M. Sarmis:** None. **M. Baudry:** None. **S. Bischoff:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.18/U12

Topic: C.08. Epilepsy

Support: NIH/NINDS Grant 5R01 NS039943-8

IMSD/NIH Grant 5R25 HL108853-02

Title: The behavioral rescue with rapamycin early following status epilepticus is not long-lasting

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Abstract: RATIONALE: Status epilepticus (SE) is associated with the development of epilepsy and comorbidities, including deficits in cognition. Previous studies have implicated the mammalian target of rapamycin (mTOR) signaling cascade in the pathophysiology of SE and epilepsy using the mTOR complex 1 (mTORC1) inhibitor rapamycin (Rap). Under basal conditions, mTORC1 regulates protein synthesis and is an important mediator of synaptic plasticity. We have recently shown that aberrant mTOR signaling contributes to behavioral deficits that occur 2 weeks following SE. In the studies reported here, we determined whether aberrant mTORC1 signaling plays a role in hippocampal-dependent learning and memory tasks, locomotion, and anxiety in the epileptic animal and whether early rapamycin rescue is long-lasting.
Methods: SE was induced in rats using pilocarpine (SE) while controls (Sham) received saline. Two weeks after SE induction, we administered Rap or vehicle (Veh) every other day for one week. The treatment groups were Sham+Veh, Sham+Rap, SE+Veh, and SE+Rap (n=7-12 per group). We tested SE and Sham animals (from each treatment group) in the Morris Water maze (MWM), Novel Object Recognition (NOR), and the Open Field task (OF) tasks. To determine whether early Rap was long lasting, we tested animals 5 months after treatment (n=6 per group). After behavioral testing was completed, we performed western blotting to verify inhibition of mTORC1 (S6 phosphorylated at S240/244).
Results: SE+Veh rats exhibited significantly longer escape latencies during the acquisition phase in MWM test and decreased time spent in the target quadrant searching for a hidden platform compared to Sham+Veh animals ($p< 0.05$ and $p<0.01$, respectively). In NOR, SE+Veh animals displayed no preference for the novel object as compared to controls ($p<0.001$). In the SE+Rap group, the deficits in MWM and NOR were rescued to performance levels of Sham+Veh animals. SE+Veh rats spent more time in the inner portion of the OF arena than Sham + Veh and

displayed increased distance traveled and travel velocity ($p<0.05$). When tested 5 months after SE, early Rap treatment did not attenuate any hyperactive locomotion in OF nor did early Rap treatment rescue any deficits in MWM or NOR (p-values not significant). For all behavioral tasks, Rap had no effect on Sham + Rap animals.
Conclusions: These data suggest that aberrant mTORC1 signaling contributes to deficits in hippocampal-dependent learning and memory. Future studies are planned to evaluate whether late mTORC1 inhibition may rescue these deficits in animals in which epilepsy has already been established, particularly since the early treatment is not long lasting.

Disclosures: A. Carter: None. **A.L. Brewster:** None. **J.N. Lugo:** None. **W.L. Lee:** None. **A.E. Anderson:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.19/U13

Topic: C.08. Epilepsy

Support: NINDS (grant #1U54NS079202-01, DZ, AD CW, HW, BI, BH, PJL, MAR)

Title: Anticonvulsant activity of a parenteral allopregnanolone formulation in mouse seizure and status epilepticus models

Authors: *D. ZOLKOWSKA[1], A. DHIR[1], C. WU[1], H. WULFF[2], B. INCEOGLU[3], B. D. HAMMOCK[3], P. J. LEIN[4], M. A. ROGAWSKI[1];
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Abstract: Allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 5α,3α-P) is an endogenous progesterone-derived neuroactive steroid that is positive allosteric modulator of synaptic and extrasynaptic GABAA receptors. 5α,3α-P exerts anticonvulsant activity in diverse animal seizure models, including models of status epilepticus (SE). SE is a neurological emergency requiring rapid treatment, typically by the IV (intravenous) or IM (intramuscular) routes. Here we characterized the activity of a parenteral 5α,3α-P formulation in the 6 Hz and PTZ seizure models, and in a new SE model. 5α,3α-P was formulated in 6% (0.5 and 1.5 mg/ml) and 24% (6 mg/ml) sulfobutylethers β-cyclodextrin sodium salts (Captisol®) in 0.9% saline. 5α,3α-P was injected IV or IM in mice prior to administration of a 6 Hz electrical stimulus or PTZ (80 mg/kg, IP). In the 6 Hz seizure model, 5α,3α-P in doses of 0.5 and 1.5 mg/kg IV conferred seizure

protection in 50% and 100% of animals at 1 min after dosing. The protective effect declined rapidly and was no longer evident at 15 and 30 min after the IV bolus with the low and higher doses, respectively. 5α,3α-P IV at doses as low as 0.25 mg/kg significantly delayed the onset of PTZ-induced seizures. 5α,3α-P IM was similarly effective in the 6 Hz model: 1.5, 3 and 6 mg/kg doses protected 50%, 90% and 100% of animals, respectively. Seizure protection peaked at 5-10 min and persisted for up to 60 min. 5α,3α-P IM at doses as low as 0.5 mg/kg significantly delayed the onset of PTZ-induced seizures in mice. SE was induced with tetramethylenedisulfotetramine (TETS; 0.2 mg/kg IP) followed by riluzole (10 mg/kg IP). After 30 min of SE, mice were treated IM with 5α,3α-P or vehicle and observed for 1 h. All surviving animals were checked daily for 7 days. 5α,3α-P IM at dose of 3, 6 and 12 mg/kg terminated SE within 1 h and prevented mortality in 67%, 67% and 83% of animals, respectively. In conventional mouse seizure models, 5α,3α-P is highly potent when administered IV but it has a short duration of action; the onset of action is modestly delayed and the duration of action is prolonged with IM administration. 5α,3α-P is also effective in terminating seizures and maintaining survival following persistent seizure activity. These studies support the potential of 5α,3α-P in the treatment of SE.

Disclosures: D. Zolkowska: None. **A. Dhir:** None. **C. Wu:** None. **B. Inceoglu:** None. **B.D. Hammock:** None. **P.J. Lein:** None. **M.A. Rogawski:** None. **H. Wulff:** None.

Poster

534. **Epilepsy: Drug Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 534.20/U14

Topic: C.08. Epilepsy

Support: USAMRAA W81XWH-11-2-0026

Title: Characterization of methamphetamine’s effect on post-traumatic epilepsy

Authors: D. SMITH, D. BROOKS, E. WOHLGENHAGEN, T. RAU, *D. J. POULSEN; Dept Biomed & Pharmaceut. Sci., Univ. Montana, MISSOULA, MT

Abstract: We have shown that low-dose methamphetamine significantly improves cognitive and behavioral outcomes following severe TBI. We hypothesized that methamphetamine might also protect against post-traumatic seizures. In this study, we used the well-established and clinically relevant rat lateral fluid percussion injury model to induce severe TBI. At 36 hours after injury, cortical tissue from controls and methamphetamine treated groups were analyzed via gene array. Interestingly, methamphetamine significantly altered the expression of several genes previously

implicated in seizure activity, including those involved in neuroinflammation and blood-brain-barrier integrity. These data support the concept that methamphetamine may prevent or reduce post-traumatic seizures.
We conducted immunohistochemical analysis to further examine the potential of methamphetamine to reduce post-traumatic seizures. TBI causes the loss of parvalbumin and somatostatin positive inhibitory interneurons. We have observed a significant loss of parvalbumin and somatostatin positive cells within the cortex and hippocampus at 46 days following severe TBI. We have also observed a significant loss of the glutamate transporter, EAAT2 within the cortex and hippocampus at 46 days pot injury. This loss of inhibition and enhanced excitatory signal potential can promote seizure activity. Methamphetamine treatment does not appear to preserve inhibitory interneurons nor prevent the loss of EAAT2. We are currently conducting additional analysis to investigate other seizure-associated proteins identified by gene array.
Video/EEG recordings were also captured to characterize epileptiform wave patterns and clinical seizure behavior following severe TBI. Preliminary results indicate an evolution of epileptic activity following TBI characterized principally by interictal spikes, and myoclonic seizures. Our preliminary results suggest that methamphetamine does not alter spontaneous seizure development within 2 months of injury. Additional experiments, which incorporate a PTZ challenge, are underway to determine if methamphetamine treatment alters seizure susceptibility. Data from these studies will help to identify critical changes that occur after TBI and focus efforts on those that make significant contribution to post-traumatic epilepsy.

Disclosures: D. Smith: None. **D.J. Poulsen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sinapis Pharma. **D. Brooks:** None. **T. Rau:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sinapis Pharma. **E. Wohlgenhagen:** None.**Poster**

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.01/U15

Topic: C.08. Epilepsy

Title: Epilepsy and consciousness: Behavioral deficits in partial seizures are bimodally distributed

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Abstract: Impaired consciousness in epilepsy has a significant negative impact on patient quality of life, yet the mechanisms for loss of consciousness during seizures are poorly understood. In this study, we explore whether impaired consciousness during partial seizures can usually be attributed to specific deficits in the content of consciousness or to a more general decrease in the overall level of consciousness. Prior studies concerning loss of consciousness during seizures have relied on retrospective and subjective methods. We recently developed the responsiveness in epilepsy scale (RES), a prospective tool for assessing ictal behavior that provides objective data about specific deficits in cognition and sensorimotor function. Here we examine the results of RES testing in 83 partial seizures captured in 30 patients. All patients were recruited during admission to Yale New Haven Hospital, and were undergoing continuous video/EEG (cVEEG) for seizure evaluation at the time of enrollment. All seizures were scored on a scale from 0 to 4, and performance for partial seizures were analyzed based on the initial cycle of ictal testing. We find that RES scores tend to be bimodally distributed, such that scores of 0 (no response) and 4 (unimpaired response) occur most frequently for all RES items. Furthermore, 75 of 83 partial seizures tested receive a score of 0 or 4 on the first RES question administered, and this initial performance predicts impairment on subsequent items. These distinct patterns of impairment correlate with those seen in “simple partial” and “complex partial” seizures. To determine whether the bimodal distribution of RES scores might be related to bias intrinsic to the scale, we also examined results of standardized testing in a cohort of brain-injured patients. In this comparison group, responsiveness was assessed using the JFK Coma Recovery Scale_Revised (CRS-R), a validated tool for assessing the minimally conscious state from which the RES was derived. We analyze the score distributions for the CRS-R subscales used to derive most RES items, and compare these results to RES scores in partial seizures. We find that scores in brain-injured patients are not bimodal, and are more evenly distributed than in partial seizure patients, suggesting that the bimodal nature of RES scores is not a result of scale bias but may be a finding unique to partial seizures. Our results indicate that partial seizures can often be cleanly separated into those with vs. without overall impaired responsiveness. These findings suggest that impaired level of consciousness affecting overall behavioral arousal in partial seizures may be crucial rather than selective deficits.

Disclosures: C. Cunningham: None. **W. Chen:** None. **A. Shorten:** None. **T. Choezom:** None. **M. McClurkin:** None. **C. Schmidt:** None. **A. Bozik:** None. **C. Best:** None. **M. Chapman:** None. **V. Chu:** None. **M. Furman:** None. **J.T. Giacino:** None. **H. Blumenfeld:** None.

Poster

535. Epilepsy: Human Studies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.02/U16

Topic: C.08. Epilepsy

Title: Spatiotemporal evolution of seizures using a 3D object detection algorithm

Authors: *R. WAHNOUN, A. BHARGAVA, P. D. ADELSON;
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Abstract: Approximately 65 million people worldwide are living with epilepsy. While some cases can be controlled with anti-epileptic drugs, others require more drastic measures for alleviation. In these situations, focal resection surgery may be considered. Prior to surgery, patients must undergo a battery of tests, including electrocorticography (ECoG) under which electrodes arrays are placed on the surface of the brain for a period of 1-3 weeks. Seizures onsets are located by visually assessing pattern changes in the brain signals.
This study aims to elucidate novel information regarding seizure origin and spread from a quantitative perspective, using a combination of signal and image processing techniques on ECoG data.
It is hypothesized that the spatiotemporal spread of seizures contains information that may be useful in focal resection surgery and offers a clinical advantage over subjective expert estimation. Refining ictal localization could minimize brain tissue removal, and eventually reduce function loss.
Raw cortical data are transformed in the spectral domain, and then converted into sequences of images. These “videos” of cortical signals are then processed through an object detection algorithm to estimate and locate bursts of activity.

Disclosures: R. Wahnoun: None. **P.D. Adelson:** None. **A. Bhargava:** None.

Poster

535. Epilepsy: Human Studies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.03/U17

Topic: C.08. Epilepsy

Support: Coulter Foundation Grant

Title: Human mesial temporal lobe single neuron dynamics during recruitment into a generalizing seizure

Authors: *A. MISRA[1], X. LONG[1], M. SPERLING[2], A. SHARAN[3], K. MOXON[1]; [1]BIOMED, Drexel Univ. BIOMED, Philadelphia, PA; [2]Neurol., [3]Neurolog. Surgery, Thomas Jefferson Univ., Philadelphia, PA

Abstract: The seizures of temporal lobe epilepsy, which are associated with an increased resistance to pharmacotherapy, can be roughly classified as generalizing, (in our case spreading from neocortex to limbic structures), and non-generalizing. Recent studies of single neuron activity have found heterogeneity of the activity of neocortical neurons prior to the onset of seizures. However, animal studies in limbic structures, including those from our own lab focusing putative pyramidal cells and interneurons, demonstrate that these two populations may have distinct firing patterns. To identify similar patterns in humans, platinum-iridium micro-wires were implanted into the mesial temporal lobe of patients undergoing diagnostic evaluation at Thomas Jefferson University under approval of the university institutional review board and according to OHRP guidelines. Patients were continuously recorded (24/7) using a high-speed acquisition system (0.8Hz - 9000 Hz) (Neuralynx, Bozeman MT). Data from 9 generalizing and 9 non-generalizing seizures across 8 patients were analyzed. 105 single neurons were discriminated (Offline Sorter, Plexon, Dallas TX) during preictal periods (two hours terminating in seizure onset) and 44 during interictal periods (more than 12 hours from seizure onset). Population peri-event time histograms were created by aligning each seizure to its onset time as determined clinically, binning the data into 2 second bins and averaging the firing rate in each bin for all neurons across all seizures. In a similar manner, normalized unit-field coherence spectrograms were created. For non-generalizing seizures there were no changes in firing rate or unit-coherence for either pyramidal cells or interneurons. For generalizing seizures, pyramidal cell activity increased within seconds of seizure onset with no measurable change in unit-field coherence. Interneurons, on average, showed a significant decrease in firing rate starting approximately 1 minute prior to seizure onset. Moreover, similar to our data from animal studies, there was an average decrease in unit-field coherence from 1-8 Hz in the 10 minutes prior to seizure. On a per-neuron level this pattern of activity was present in 50% of recorded interneurons across 75% of generalizing seizures. These data suggest that seizure spread through white matter tracts, similar to seizure spread within cortical structures, may depend on a break-down of inhibition in recruited structures.

Disclosures: A. Misra: None. **X. Long:** None. **M. Sperling:** None. **A. Sharan:** None. **K. Moxon:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.04/U18

Topic: C.08. Epilepsy

Support: OTKA PD101754

TÁMOP-4.2.1./B-11/2/KMR-2011-002

TÁMOP-4.2.2./B-10/1-2010-0014

TÉT-Multisca

TÉT-Neurogen

FP7-Neuroseeker

OTKA K81354

Title: Spatial variability of cortical ripples in humans

Authors: *E. TÓTH[1,2], L. ENTZ[3], I. ULBERT[2,1], L. ERőSS[3,1], D. FABÓ[3];
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Abstract: Background
Based on animal and human research ripple oscillations (80-300Hz) participate in physiological processes and epileptiform transformations in the brain. The cortical generators of such fast oscillations were demonstrated to have generators limited to a small cortical area. The purpose of this study is to describe the spatial variability of ripple oscillations and demonstrate that wide cortical area can contribute to their generation.
Methods
Epilepsy patients undergoing invasive preoperative evaluation were selected in this study. Two patients had focal cortical dysplasia in the right postcentral gyrus, and had therapy resistant elementary motor seizures. The central area was covered by subdural grid electrodes. Ripple oscillations were detected on slow wave sleep EEG segments using semi automated method, first detecting putative ripple events (PR) exceeding 5 standard deviation (SD) of band pass and root mean square filtered data, and thereafter visual selection was performed.
Results
The majority of PRs appeared on 1 or 2 channels, but there was a significant proportion of the events presented on various number of channels, in some cases almost all channels. Wide range ripples were found more often in the proximity of the seizures. In this case bimodal distribution was observed in the involved channel number histogram.

Discussion
We demonstrated that relatively large cortical areas may contribute to the genesis of ripple oscillations, that area might vary significantly. Two populations of small and wide range ripples were demonstrated. We hypothesize that the cortical extent of the ripples may be a factor of it's epileptogenic nature.

Disclosures: E. Tóth: None. **L. Entz:** None. **I. Ulbert:** None. **L. Erőss:** None. **D. Fabó:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.05/V1

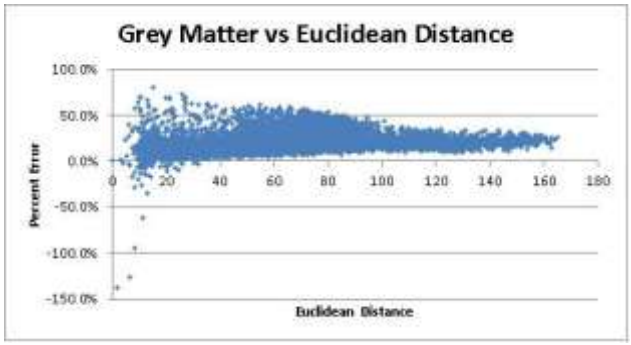
Topic: C.08. Epilepsy

Title: Accurate estimation of cortico-cortical distance between intracranial electrode contacts

Authors: *J. D. TURNER[1], B. JOSHI[1], A. PANDAY[1], R. MUNBODH[2], H. P. ZAVERI[3]; [1]Electrical and Computer Engin., UNC Charlotte, Charlotte, NC; [2]Radiation Oncology, Univ. of Pennsylvania, Philadelphia, PA; [3]Neurol., Yale Univ., New Haven, CT

Abstract: Objective: Intracranial EEG (icEEG) inter-contact distance, which is important for measurements of brain relationship and estimates of time-delay, is commonly estimated as the Euclidian distance between the centers of two icEEG contacts. We propose a more accurate method to calculate the distance between icEEG contacts.
Methods: Intracranial EEG electrode contacts placed in 22 adult patients being monitored for epilepsy surgery were accurately located from post-implant CT and co-registered to a pre-implant MRI scan and the MRI of a standard brain. The MR images were rendered as a 3D surface. Cortico-cortical distances along the brain surface were calculated using a variant of Dijkstra's algorithm, a common shortest-path algorithm. The surface distance estimates were compared to the Euclidean distance.
Results: The surface distance estimates were significantly greater than the Euclidean estimates and can generally be approximated as being 30% longer. The largest discrepancy between surface and Euclidean distances was observed for distances between 4.5cm and 8cm. Difficulties resulted from inaccurate location of icEEG contacts on the brain surface, and previous resective surgery.
Discussion: A considerable difference between brain surface and Euclidean distances was observed. In its current form, the algorithm takes nearly 20 hours to complete analysis of a single patient, while Euclidean distances can be calculated in real time. Ongoing work involves

increasing the speed of the algorithm and addressing the challenges listed above.
Conclusions: The Euclidean distance metric can considerably underestimate the distance between two icEEG electrode contacts. This approximation can adversely affect estimates of brain relationship and time-delays. A more accurate distance measure is possible through the application of Dijkstra's algorithm to the brain surface rendered from the MRI of the patient brain.



Disclosures: J.D. Turner: None. **B. Joshi:** None. **A. Panday:** None. **R. Munbodh:** None. **H.P. Zaveri:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.06/V2

Topic: C.08. Epilepsy

Title: Immunolocalization of metallothionein in patients with temporal lobe epilepsy

Authors: *M. MENDEZ-ARMENTA[1], C. NAVA-RUIZ[1], M. ALONSO-VANEGAS[2], M. BUENTELLO-GARCÍA[2], D. JUAREZ-REBOLLAR[1];
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Abstract:
Temporal lobe epilepsy (TLE) is the most prominent example of acquired and frequent epilepsy which is commonly preceded by an initial brain injury such as an episode of prolonged seizures or status epilepticus (SE), childhood febrile seizures, hypoxia or trauma and becomes pharmacologically untreatable in a high percentage of patients. Two main types of TLE are generally recognized, mesial temporal lobe epilepsy which arises in the hippocampus, parahippocampal gyrus and amygdala, and lateral temporal lobe epilepsy which arises in the neocortex. On the other hand, there are four isoforms of metallothionein (MT-I - IV), of which

MT-I and -II are the best characterized, in the central nervous system (CNS), MT-I+II are expressed coordinately and are increased in macrophages/microglia and astrocytes during various inflammatory and pathologic conditions including human neurodegenerative disorders, brain injury, oxidative stress, neurodegeneration, heavy metals, and epileptic seizures.The MTs occur in several brain regions and may serve as neuroprotective proteins against reactive oxygen species causing oxidative damage and stress, ionizing radiation, or anti-cancer drugs. The main aim of this work was to describe the immunohistochemical localization of MT in the specimens derived from the patients affected by MTLE. Samples were collected of patients with TLE, patients were submitted to the protocol set out by the Epilepsy Surgery Program of the INNN in Mexico, which comprises an extensive pre-surgical clinical evaluation, video-electroencephalogram (EEG) record, magnetic resonance maging (MRI) and single photon emission computed tomography (SPECT). NeuN, GFAP, and MT antibodies were used for immunohistochemical analysis. Histopathological examination showed NeuN immunopositive cells that were analyzed for determinate the neuronal density in hippocampal and parietal cortex samples. Increased reactive astrogliosis in CA2 and CA3 of hippocampus was observed and increase MT immunoreactivity was also observed in cell and nueropil of TLE samples. In conclusion, an increased in MT expression was observed in samples from TLE patients, this is important due to MTresponse in brain damage associated to seizures and these results suggest that further studies are needed to investigate the possible neuroprotective role of MT on TLE.

Disclosures: M. Mendez-Armenta: None. **C. Nava-Ruiz:** None. **M. Alonso-Vanegas:** None. **M. Buentello-García:** None. **D. Juarez-Rebollar:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.07/V3

Topic: C.08. Epilepsy

Support: J010.0170/2010)

ETT577/2006, RET67/2005)

Title: Inflammatory process in neocortex of patients with refractory epilepsy

Authors: *J. VILLEDA, SR[1], M. ALONSO[2], L. ROCHA[4], S. OROZCO[5], V. CAMPOS[3], F. FERNANDEZ[1];
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Abstract: Objective: In this study we evaluated the expression of cytokines in patients with refractory temporal lobe epilepsy and cortical dysplasia.
Methods: We studied 13 patients (5 women and 8 men), 7 with refractory epilepsy and 6 different types of tumors with an average age of 30.6, with refractory temporal lobe epilepsy, All patients were studied by preoperative protocol standardization and temporal lobectomy candidates and amygdalohippocampectomy. The expression and distribution of TNF, IL-1β, IL-6 and COX were studied immunohistochemically in regions T1, T2, T3.
Results: We found disorganization of cortical architecture, neuronal loss, starch bodies, spongiosis, apoptotic cells, hypertrophic, dysplastic and dysmorphic neurons, ballon cells with atypical nuclei, cytoskeletal disruption in dense fibrillar aggregates, the cortical dysplasia 69.23% type IIA, and the 30.77% type IIB, numerous fibrillary astrocytes, were observed, the majority of patients have extensive cell death in the cortex, TNF-α, IL-1β, IL-6 were significantly increased in patients with epilepsy Conclusions: Our results suggest that these structural lesions and the inflammatory events will be casually associated, as well as also we found a relation with the type dysplasia, in these patients with refractory epilepsy, these findings support the idea that neuroinflammation is oriented to activation of the microglia and the astrocytes by reduce neurological morbidity and seizure control in these patients.

Disclosures: J. Villeda: None. **M. Alonso:** Other; Neurosurgery. **L. Rocha:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); research. **S. Orozco:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); research. **V. Campos:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); research. **F. Fernandez:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); research.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.08/V4

Topic: C.08. Epilepsy

Support: CONACYT grant 98386

scholarship 333114

Title: Evaluation of the density and signaling of histamine H3 receptors in the temporal cortex and hippocampus of patients with pharmacoresistant temporal lobe epilepsy

Authors: *I. BAÑUELOS-CABRERA[1], M. CUELLAR-HERRERA[2], S. OROZCO-SUAREZ[3], M. ALONSO-VANEGAS[4], J. ARIAS-MONTAÑO[1], L. ROCHA[1];
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Abstract: Temporal lobe epilepsy (TLE) is the most common type of epilepsy and becomes pharmacologically untreatable in a high percentage of patients. In TLE associated with mesial sclerosis (MTLE), the hippocampus is the epileptic focus, while the temporal cortex is involved in the propagation of epileptic seizures to other brain areas. Epilepsy involves changes in several neurotransmitters. Concerning the histaminergic system, several studies in seizure and epilepsy models suggest that histamine may exert anti- or pro-convulsant effects, the latter presumably mediated by the H3 receptor. The present study was carried out to determine the density and signaling of H3 receptors in the hippocampus and temporal cortex of 10 patients with pharmacoresistant MTLE. Tissue samples were obtained immediately after surgical resection. Results were compared with those obtained from 6 autopsies. Cell membranes were obtained from tissue samples by homogenization and centrifugation and used to determine the binding of N-α-[methyl-3H] histamine ([3H]-NMHA), a selective H3 receptor agonist. Activation of G proteins subsequent to stimulation of H3 receptors by the selective agonist immepip was also investigated by [35S]-GTPγS binding assay. For this assay, values of maximal stimulation (Emax) and half-effective concentration (EC50) were determined by non-linear regression (GraphPad Prism 5.0). Protein contents were determined according to the method of Lowry. The values of specific [3H]-NMHA binding, Emax and basal [35S]-GTPγS binding obtained from patients with MTLE were expressed as the percentage change with respect to the values obtained from autopsies. Specific [3H]-NMHA binding showed no significant changes in density of H3 receptors in the hippocampus (51%, $p>0.05$) and temporal cortex (28%, $p>0.05$) of patients with MTLE. In the hippocampus of patients with MTLE, the [35S]-GTPγS binding assay revealed no changes in the values of Emax (28%, $p>0.05$) and EC50 (from 2.2 to 1 nM, $p>0.05$). The temporal cortex of patients with MTLE demonstrated no changes in EC50 (from 1.3 to 1.5 nM, $p>0.05$), but increased Emax values (121%, $p<0.01$), an effect probably associated to high basal [35S]-GTPγS binding (177%, $p<0.05$). Our present data provide evidence that in the temporal

cortex of patients with pharmacoresistant MTLE there is an alteration in the signaling of the H3 receptor.

Disclosures: I. Bañuelos-Cabrera: None. **M. Cuellar-Herrera:** None. **S. Orozco-Suarez:** None. **M. Alonso-Vanegas:** None. **J. Arias-Montaño:** None. **L. Rocha:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.09/V5

Topic: C.08. Epilepsy

Support: C. G. Swebilius Trust

Title: Co-localized measurements of intracranial EEG spikes and extracellular glutamate in patients with medically intractable epilepsy

Authors: N. GANESH[1], C. ONG[2], C. HALDEMAN[2], E. DAMISAH[3], I. I. GONCHAROVA[1], D. D. SPENCER[3], T. EID[2], *H. ZAVERI[1];
[1]Neurol., [2]Lab. Med., [3]Neurosurg., Yale Univ., New Haven, CT

Abstract: Purpose: Interictal spikes are considered to be a marker of excitability in the epileptic brain, though recent work by us and others has questioned whether spikes more truly reflect inhibition rather than excitation. We have also reported that basal levels of extracellular glutamate are increased in the epileptic brain. Here we sought to correlate co-localized measurements of interictal spikes and basal extracellular glutamate concentrations in patients undergoing intracranial EEG (icEEG) monitoring for epilepsy surgery.
Methods: A total of 20 patients were included in this study. Brain microdialysis measurements of glutamate and glutamine obtained from 59 combined depth EEG electrode/microdialysis catheters (Spencer probes) were analyzed. Spike counts were documented for the two icEEG contacts on either side of the microdialysis membrane, all contacts within 3cm of each probe, and all contacts within the region where the probe was located. Additionally, basal glutamate and basal glutamine values were correlated with the distance of the probe from the seizure onset area.
Results: The basal glutamate and basal glutamine values were negatively correlated with the probe distance to seizure onset area with correlation coefficient values of 0.2 ($p=0.06$) and 0.4 ($p=0.001$), respectively. We observed a slightly negative correlation between the basal glutamate values and spike counts. The correlation coefficient values for the probe contacts, contacts within 3cm, and regional contacts were 0.07, 0.08, and 0.14, respectively. In areas of high spiking, basal glutamate values were particularly low.

Discussion: The higher basal glutamate and glutamine values closer to the seizure onset area suggest a correlation between increased glutamate and glutamine levels and the seizure onset area. Spikes are traditionally though to indicate brain excitation. The slight negative correlation observed between basal glutamate level and spike counts, however, support the suggestion that spikes may instead be a measure of brain inhibition.
Conclusions: This study suggests considerable value for co-local *in-vivo* measurements of electrophysiology and neurochemistry to improve our understanding of the two modalities and the seizure onset area in patients with medically intractable epilepsy.

Disclosures: N. Ganesh: None. **C. Ong:** None. **C. Haldeman:** None. **E. Damisah:** None. **I.I. Goncharova:** None. **D.D. Spencer:** None. **T. Eid:** None. **H. Zaveri:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.10/V6-DP5

Topic: C.08. Epilepsy

Support: Burroughs Wellcome Fund

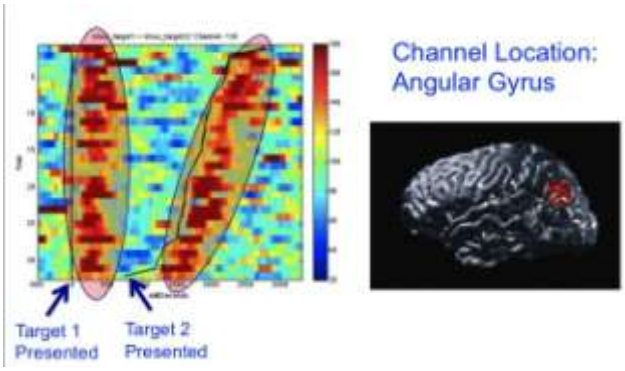
National Science Foundation

Title: Intracranial depth electrode recordings with fine spatial and temporal resolution show neural correlates of movement in humans

Authors: M. KERR[1,2], H.-J. PARK[2], K. KAHN[1], J. BULACIO[2], J. GONZALEZ-MARTINEZ[2], *S. V. SARMA[1], J. GALE[2];
[1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Clin. Neurosci., Cleveland Clin., Cleveland, OH

Abstract: Most neural data collected from humans takes the form of electroencephalogram (EEG) or functional magnetic resonance imaging (fMRI). EEG provides high temporal resolution but poor spatial resolution, while fMRI provides high spatial resolution but low temporal resolution. To achieve both high temporal and spatial resolution, electrodes in the various brain structures are usually implanted in animal subjects. However in a small fraction of patients with refractory epilepsy, intracranial depth electrodes precisely guided by stereotaxis, a procedure known as stereoelectroencephalography (SEEG), are implanted as a means of identifying the epileptogenic area. SEEG recordings include tens to hundreds of channels that are distributed in deep and peripheral brain structures and therefore have fine spatial and temporal

resolution. This gives us a unique opportunity to study the human brain and neural correlates of behavior.
In this preliminary study, a patient implanted with 12 electrodes (113 channels), spatially distributed over cortex and deep structures, was asked to perform a joystick based motor task. The task involved moving a cursor to two consecutively presented circular targets and then pressing a button when cued. A measure of high frequency activity (HFA) was used as a proxy for neural activity in the region around each channel. HFA was calculated by averaging the normalized power in each 10 Hz band between 50 and 150 Hz.
Data show clear trial-by-trial activity modulations responding to new target presentation and task type instructions (angular gyrus), button-pressing (insula), and reward (middle temporal gyrus). For example, whenever a new movement target is presented, HFA modulated in the angular gyrus and robustly increased by approximately 80% from trial baseline with the effect lasting around 400 ms, supporting previous work implicating angular gyrus in shifts in spatial attention. These results demonstrate the achievable spatial and temporal resolution of SEEG combined with a behavioral task but also provide unique insight into the function of human brain.



Disclosures: M. Kerr: None. **H. Park:** None. **K. Kahn:** None. **J. Bulacio:** None. **J. Gonzalez-Martinez:** None. **S.V. Sarma:** None. **J. Gale:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.11/V7

Topic: C.08. Epilepsy

Support: AG12411

Windgate Foundation

Title: Neuronal stress responses are more related to APOE genotype than to stress modality or age

Authors: O. ABOUD[1], R. E. MRAK[2], F. A. BOOP[3], *S. T. GRIFFIN[1,4];
[1]Geriatrics, Univ. Ark Med. Sci., LITTLE ROCK, AR; [2]Pathology, Univ. of Toledo Hlth. Sci. Campus, Toledo, OH; [3]Neurosurg., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [4]Grecc, Central Arkansas Veterans Hlth. Care Syst., Little Rock, AR

Abstract: Precocious development of Alzheimer-type neuropathological changes in epilepsy patients, especially in *APOE* ε4 carriers is well known, suggesting the importance of investigating epilepsy-related neuropathological changes as they relate to each of the possible *APOE* allelic combinations. Frozen and paraffin-embedded tissue samples were resected from superior temporal lobes of 92 patients undergoing temporal lobectomies as a treatment for medication-resistant temporal lobe epilepsy (3 months- 73 years old, representing each of the *APOE* allelic combinations). Tissue samples were analyzed using immunofluorescence histochemistry, western immunoblot, and real-time PCR to determine genotype effects on neuronal number and size, neuronal and glial expressions of amyloid β (Aβ) precursor protein (βAPP), apolipoprotein E (ApoE), Aβ, S100B, interleukin-1α and β, and α and β secretases, and on markers of neuronal stress, including DNA/RNA damage and caspase 3 expression. Allelic combinations of *APOE* influenced each epilepsy-related neuronal and glial response measured. Patients with *APOE* ε3,3 genotype had larger neurons with less DNA fragmentation than other genotypes and greater numbers of activated IL-1-immunoreactive glia per neuron, suggesting neuronal resilience against stress. However, this resilience may foster plaque burden—25 % of those with *APOE* ε3,3 genotype (n=53) had plaques; no patient with even one *APOE* ε2 allele (n=15) had Aβ plaques, and neuronal size in patients having an *APOE* ε2 allele was, like those with *APOE* ε3,3 larger than in other genotypes. *APOE* ε4,4 conferred the weakest neuronal resilience in epilepsy. To determine if these epilepsy-related, *APOE* genotype dependent changes are similar to those in another neurological condition, analogous tissue samples from 10 autopsy-verified Alzheimer brains, and from 10 neurologically and neuropathologically normal (control) patients were analyzed with regard to neuronal size. As in epilepsy, Alzheimer patients with *APOE* ε4,4 had smaller neurons than did those with *APOE* ε3,3. Interestingly, there was no *APOE* genotype-dependent difference in these parameters in neurologically normal patients. Our findings provide evidence that the strength of the neuronal stress response is more related to patient *APOE* genotype than to either the etiology of the stress or to the age of the patient and are consistent with the idea that hyperexcitation elicits compensatory responses aimed at neuron repair and survival; but when chronic, these events may give rise to the precocious appearance of neuropathological changes, suggesting that *APOE* genotyping may be a useful tool in treatment decisions.

Disclosures: O. Aboud: None. **S.T. Griffin:** None. **R.E. Mrak:** None. **F.A. Boop:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.12/V8-DP4

Topic: C.08. Epilepsy

Title: Detecting preseizure states in intracranial EEG data using an adaptation of diffusion maps

Authors: *D. DUNCAN, R. TALMON, H. P. ZAVERI, R. R. COIFMAN;
Yale Univ., New Haven, CT

Abstract: Rationale: We studied the variability of the statistics of the icEEG data of patients with epilepsy over time to distinguish between different states of a patient. Diffusion mapping, which is one of the leading manifold learning methods, provides dimensionality reduction of the data as well as pattern recognition that can be used to distinguish different states of a patient. A new algorithm, which is an adaptation of diffusion maps, is developed to construct coordinates that generate efficient geometric representations of the complex structures in the icEEG data. Moreover, the combination of local covariance matrices and the Mahalanobis distance in the algorithm is used to remove the noise from the data to extract the underlying brain activity.
Methods: Intracranial EEG data were collected from a patient with localization related epilepsy who was undergoing presurgical evaluation at the Yale-New Haven Hospital. The seizure onset area was located on the right occipital lobe. We focus on the 3 electrode contacts that overlaid the seizure onset area and use our algorithm to distinguish interictal and preseizure states from 7 seizure episodes.
Results: Numerical results show that the proposed approach provides a distinction between interictal and preseizure states by constructing a mapping that separates data along these two classes. Once we have learned the mapping, it can quickly be applied to new data for classification in real time.
Conclusions: Based on the variability of the statistics of the data, we were able to show a distinction between interictal and preseizure states. The combination of the local statistics and the Mahalanobis distance is shown to be beneficial for such noisy data. Our goal is to define a threshold that separates the interictal state from the preseizure state in a wide range of cases and develop an automatic method to predict seizures.

Disclosures: D. Duncan: None. **R. Talmon:** None. **H.P. Zaveri:** None. **R.R. Coifman:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.13/V9

Topic: C.08. Epilepsy

Support: William M Keck Foundation

NIH Grant T32 GM080842

NIH Grant T32 GM008185

NIH Grant R33 DA026109

UCLA Department of Biomathematics

Title: Predicting when MRI and FDG-PET will exhibit epileptogenic findings

Authors: *W. T. KERR[1], A. TREFLER[2], K. R. RAMAN[2], E. S. HWANG[2], N. SALAMON[3], M. S. COHEN[4];
[1]UCLA Semel Inst., Los Angeles, CA; [2]Psychiatry, [3]Neurology, Radiology, [4]Psychiatry, Neurology, Biomed. Engineering, Radiological Sciences, Biomed. Physics, UCLA, Los Angeles, CA

Abstract: The seizure onset zone (SOZ) of uncontrolled or medication resistant seizures epilepsy (MRE) has been associated with congenital or disease-initiating structural and/or metabolism abnormalities as well as progressive changes. The progressive changes, most commonly, are focal atrophy, MRI T2 signal hyperintensity and progressive focal hypometabolism on FDG-PET. These progressive changes reflect cell death of inhibitory neurons, gliosis and metabolic dysfunction within neurons. The majority of patients have no observable changes after their first seizure. Conversely, roughly 75% of patients with long-standing MRE have observable structural and/or metabolic abnormalities that help localize their SOZ. This results in a clinical conundrum: when should neuroimaging be acquired from patients with seizures? In this work, we developed a quantitative estimate of the pre-test probability that neuroimaging will reveal a potentially diagnostic abnormality. We used a Bayesian logistic regression model based upon the duration of seizure disorder, seizure frequency, age, gender and the etiology and location of the seizures from over 450 patients with medication resistant epilepsy admitted for long term video-EEG monitoring. Our model confirms that the observed probability that an abnormality was present increased logarithmically with duration of the seizure disorder (95% CI odds ratio MRI 1.09-1.44, PET 1.01-1.88 per log year) and a definitive diagnosis of temporal lobe epilepsy (95% CI odds ratio MRI 1.18-3.09, PET 1.82-5.51), controlling for the other included factors. Interestingly, when compared to patients with non-epileptic seizures, there was no significant evidence that patients with extratemporal epilepsy were more likely to exhibit structural or metabolic abnormalities (95% CI odds ratio MRI 0.75-

1.79, PET 0.40-1.46). The lack of effect of seizure frequency compared to duration of seizure disorder suggests that the characteristic cell death, gliosis and metabolic dysfunction was not associated with the number of seizure events, but instead with the chronic propensity to have these events. While our primary research aim was to improve the cost-benefit ratio of expensive clinical imaging, these data also help elucidate some of the pathologic mechanism behind why resective surgery for epilepsy was more effective earlier in a patient's course of disease.

Disclosures: W.T. Kerr: None. **A. Trefler:** None. **K.R. Raman:** None. **E.S. Hwang:** None. **N. Salamon:** None. **M.S. Cohen:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.14/V10

Topic: C.08. Epilepsy

Title: Effect of parent gender and education on the quality of life in pediatric epilepsy-results of an outpatient cross sectional study

Authors: M. IQBAL[1], S. AMIRSALARI[2], *M. RAZA[3];
[1]Fac. of Medicine,, [2]Dept. of Pediatrics, [3]Section of Neurosciences, Dept. of Neurology, Fac. of Med., Baqiyatallah Univ. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract: Introduction: In developing countries lacking modernized healthcare system, quality of life (QOL) and general health (GH) of pediatric epilepsy patients depends heavily on parental care and family support. Studying factors that affect QOL can help their parents and health care providers to plan their life better. We assessed parental factors affecting QOL of pediatric epilepsy patients in outpatient setting.
Methods: A cross-sectional survey of pediatric epilepsy patients (n=91, 49 M, 42 F, age 10.2 ± 0.3 y) was carried out using US quality of life in childhood epilepsy (QOLCH) and Seizure Severity Questionnaire (SSQ). Patients and their parents were interviewed at the outpatient pediatric neurology clinic. Patients with progressive neurodegenerative disorder, severe to profound mental retardation, or visual/hearing impairment were excluded from the study. Basic demographic data, type of epilepsy, seizure frequency, severity and other characteristics and social life of patients were recorded. Relevant data were graded using Likart scale and analyzed.
Results: The most common seizure type among patients was Complex Partial Seizure (CPS, n=39, 37.4%) followed by Generalized Tonic-clonic Seizure (GTCS, n=16, 17.6%). Neither QOL nor the GH of patients was related to the type of seizure. Total QOL in male patients with a

High school level of education in father was significantly different from those whose father's level of education was MSc (2.96±.131 vs 4±.316, p<.05) but father's education had no significant impact on QOL of female patients. Mother's level of education had no significant effect on QOL of patients. The overall GH in male patients with a high school level of education in mother was significantly different from those whose mother's level of education was BSc (3.24±.125 vs 2.25±.313, p<.05). Totally also, GH was significantly different in patients with a high school level of education in mother with those whose mother had a BSc level of education (3.13±.104 vs 2.36±.225, p<.05). GH in male patients whose father had a high school level of education was significantly different from those whose father had a MSc level of education (3.21±.157 vs 2±447, p<.05). The GH and QOL in patients significantly correlated with each other (Spearman) in both male (r=.4) and female(r=.3) patients. These two factors were totally correlated with each other also. There was a significant correlation between GH and number of seizures during recent week (Spearman, r=.215).
Conclusion: Parental gender and education has significant impact on the QOL and GH of pediatric epilepsy patients. Family education about epilepsy can help epileptic patients to have a better life.

Disclosures: M. Iqbal: None. **M. Raza:** None. **S. Amirsalari:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.15/V11

Topic: C.08. Epilepsy

Support: NIH/NINDS R01 NS058802

Title: Spatial organization of the mapk signaling interactome in human epileptic brain

Authors: *S. BAGLA[1], J. LOEB[2];
[1]CMMG, Wayne State Univ., Detroit, MI; [2]Univ. of Illinois Chicago, Chicago, IL

Abstract: Epilepsy is a disease of recurrent seizures, however, the exact mechanisms and networks that produce epileptic activities in the neocortex are not known. Surgical removal of the focal brain regions that display a variety of epileptic activities presents an excellent opportunity to discover the molecular and cellular basis of human epilepsy. We have taken a high-throughput genomic approach and identified a highly consistent group of differentially expressed genes that correspond to these epileptic regions. Highly represented in regions that show high interictal spiking are genes that implicate MAPK/CREB signaling, Immediate Early

Genes (IEGs), and synaptic plasticity genes. Bioinformatic analyses showed a number of clusters within the MAPK/CREB genes that included both activators and inhibitors of MAPK signaling, raising an important question as to why both activators and inhibitors are both induced. As a means to answer this, we mapped these activators and inhibitors in human epileptic neocortical foci using in situ hybridizations and immunolabeling. One of the interesting inhibitors of MAPK/CREB that was significantly upregulated in our microarrays was DUSP4, a member of Dual Specificity Phosphatases, which acts by targeting both isoforms of ERK1/2. In situ hybridization studies of serial brain sections shows that DUSP4 is expressed in discrete micro-domains in the superficial neocortical layers. DUSP4 expression was inversely related to di-phosphoERK, phosphoCREB, EGR1, and DUSP6, suggesting that DUSP4 activation in regions of high interictal activity directly inhibits the spread of electrical activity in these focal regions. To test this, we developed a model that uses repeated depolarization of human neuronal Sy5Y cells to determine the mechanism of action of DUSP4. We found induction of DUSP4 protein within several hours of chronic depolarization, irrespective of CREB activation. These studies utilizing high throughput genomic studies from human neocortex begin to define both the mechanistic and spatial roles of MAPK signaling in neocortical epilepsy and that have the potential to produce novel therapeutics.

Disclosures: S. Bagla: None. **J. Loeb:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.16/V12

Topic: C.08. Epilepsy

Support: A partial fulfilment of the Graduate Program in Biological Sciences of the National Autonomous University of México (UNAM).

Title: Specific transcripts of the Brain Derived Neurotrophic Factor increase in hippocampus of patients with sclerosis-associated temporal lobe epilepsy

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Abstract: The Brain Derived Neurotrophic Factor (BDNF) has been strongly implicated in the maintenance of the cytoarchitecture of mammalian hippocampus. In temporal lobe epilepsy (TLE), hippocampus has been proposed as an important brain structure for the onset and propagation of electrical discharges. In TLE patients, especially in those showing hippocampal sclerosis (HS), an increment of the genetic expression and protein levels of BDNF has been reported. Moreover, in vitro and in vivo BDNF administration in the nervous tissue of rodents has been associated with the HS phenotype. Human BDNF gene has a highly complex regulated expression leading to the synthesis of at least 17 different transcripts. The aim of the present study was to evaluate the levels of BDNF transcripts I, II, IV and VI in hippocampus of TLE -HS patients in comparison to TLE associated to brain lesions (i.e. infections, tumors, cavernomas). Method: Total ARN was extracted from HS (n=12) and lesions (n=8) hippocampus samples of TLE patients after surgery. The aforementioned transcripts were evaluated by quantitative Real-Time PCR, using the TATA binding protein as the reference gene (Taqman pre-designed assays). T student test was used to compare mean between groups. Results: An increment in the expression of transcript IV (p=0.04), as well as a tendency in the same direction for exons II (p=0.08) and VI (p=0.06 was found in patient with HS compared to those with lesions. Conclusions: These results are in accordance with recent animal models and human reports, indicating that the differential expression of BDNF gene could be a molecular mechanism of relevance in TLE.

Disclosures: G. Martínez-Levy: None. **L. Rocha:** None. **M.A. Alonso-Vanegas:** None. **R.M. Buentello-Garcia:** None. **F. Recillas-Targa:** None. **R. Perez-Molina:** None. **C.S. Cruz-Fuentes:** None. **A. Nani:** None. **A. Perez-Molina:** None. **M. Briones-Velasco:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.17/W1

Topic: C.08. Epilepsy

Support: CIHR

Title: Quantifying metal distributions using synchrotron x-ray fluorescence imaging of hippocampal resected in human epilepsy surgery

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Abstract: Changes in metals such as zinc have been observed in brains following seizures. The modulation of brain metal levels have also been known to initiate seizures. The precise interaction of endogenous metals and seizures, however, is not well understood and studies have been limited in their ability to study the co-occurrence of metals within the same epileptic structures. Previously we have demonstrated the use of synchrotron x-ray fluorescence imaging (SXRF) to characterize multiple metal distributions at multiple spatial scales in human cortical tissue (n = 17) recovered after minimally invasive epilepsy surgery. In this study, we examine the distribution and co-localization of elements within hippocampal regions of seizure foci, removed during surgery. Resected hippocampal tissue from surgical cases of patients with intractable mesial temporal lobe epilepsy (MTLE) were imaged using rapid-scanning SXRF and microprobe imaging (beamlines 10-2 and 2-3) at the Stanford Synchrotron Radiation Lightsource (n=7). Complete XRF spectra were captured for each pixel, allowing for whole image construction of any non-windowed element. Using SXRF imaging we were able to ascertain co-localized metals and perform quantitative analysis on a suite of elements. SXRF imaging showed definitive structure of the molecular and pyramidal cell layers of the hippocampus with a number of metals including iron and zinc. Large iron depositions were observed in several subjects with sclerotic hippocampal tissue. Analysis is underway to determine the iron species, an indication of the physiological process or potential indications of pathological damage to brain structure. These imaging studies of local circuitry thus help identify the role of brain metals in epilepsy while increasing our understanding of healthy brain structures from the perspective of endogenous brain chemistry.

Disclosures: A. Lam: None. **L. Frutos:** None. **E.L. Ohayon:** None. **C.M. Florez:** None. **S. Mylvaganam:** None. **T. Valiante:** None. **P.L. Carlen:** None. **S.M. Webb:** None. **B.D. Kocar:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.18/W2

Topic: C.08. Epilepsy

Support: ABRC grant: ADHS13-031259

NIH grant: R01GM085237

Title: Profiles of BDNF/TrkB signaling pathways in human hypothalamic hamartoma tissues

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Abstract: Human hypothalamic hamartoma (HH) is characterized by gelastic seizures, refractory to antiepileptic drugs. Evidence suggests that the source of seizures in HH patients lies within the lesion itself. Thus, identification of the potential mechanisms of epileptogenesis within HH tissues will aid the drug development for better management of this disease. Brain-derived neurotrophic factor (BDNF) that is essential for neuronal development and maintenance has been implicated in the modulation of synaptic function and plasticity. BDNF exerts its biological effects via binding to the TrkB receptor, subsequently engaging downstream signaling cascades, such as the MAPK (mitogen-activated protein kinase), Akt (protein kinase B), and PLCγ/CaMKII (calcium-calmodulin kinase II) pathways. In this study, we evaluated the major BDNF/TrkB signaling pathways in surgically resected HH tissues (n=14) versus human hypothalamic control tissues (n=8). Our results showed that phosphorylation of TrkB at Tyr816 was enhanced in HH tissues compared to the controls (P=0.015). By examining the MAPK pathways (ERK, p38, and JNK), we found that activation of all three MAPKs was elevated in HH cases than in the controls (pERK1/2, P=0.016; p-p38, P=0.049; pJNK1, P=0.001). For the Akt pathway, our results indicated that the level of active Akt (pAkt Ser473) in HH group was higher than that in the control group ($P < 0.05$). Surprisingly, the expression of PTEN (a suppressor of Akt) was also increased in HH compared to the control ($P < 0.05$). We further examined the correlation between PTEN and active Akt levels within HH group. Interestingly, a negative correlation between them was obtained, which could resulted in a further classification of the HH cases (n=14) into two subgroups (high PTEN/low pAkt and low PTEN/high pAkt). When assessing the PLCγ/CaMKII pathway, we observed that both CaMKII expression and PLCγ1 phosphorylation (pPLCγ1 Ser1248) levels were higher in HH than in the control group (CaMKII, P=0.004; pPLCγ1, P=0.00002). Finally, real-time RT-PCR results demonstrated an approximately 2.5-fold increase in BNDF mRNA expression in HH versus control tissues. Collectively, our findings suggest that multiple BDNF/TrkB signaling cascades are activated within HH lesion, which may independently and/or collaboratively contribute to epileptogenesis.

Disclosures: S. Semaan: None. **Y. Huang:** None. **J. Wu:** None. **Y. Chang:** None.

Poster

535. Epilepsy: Human Studies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.19/W3

Topic: C.08. Epilepsy

Title: Operant processes suppress epileptic processes

Authors: *J. C. NEILL[1], N. ALVAREZ[2];
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Abstract: What environmental events change epileptic processes? Earlier work from my lab demonstrated that prompts and rewards for active behavior in the everyday environment were correlated with decreases in the duration and frequency of epileptiform EEG and seizures in subjects with intractable generalized epilepsy and intellectual disability. Hypothetically, this effect was due to operant conditioning of behavior established by the everyday environment. To test this hypothesis, three subjects were monitored with concurrent EEG and video while resting, during operant conditioning and a go left-go right discrimination, and after conditioning sessions. Results from one representative subject will be discussed. During resting conditions, before operant conditioning began, the ED (percentage of time with epileptiform EEG discharges) was as high as 60%. During 5 operant conditioning sessions, in which operant responses were reinforced with variable interval schedules of reinforcement, ED was greatest (58%) before the conditioning sessions, moderate (15%) during the sessions, and minimal (4%) after the sessions. ED following sessions declined over 5 sessions of operant conditioning. When responding was stable, a sound source (pulsed white noise) was used to signal the response side producing reinforcement. In the first 12 sessions of auditory stimulus control training, control of responding increased from chance levels in session 1 to over 90% in session 12; simultaneously, during these sessions, ED was suppressed, from a high of 45% before session 2, down to 0 before session 12. During 12 sessions that it took for auditory stimulus control to reach asymptote, maximal ED was largest before conditioning sessions (45%), moderate during conditioning (25%), and lowest after training (12%). During session 12, when asymptotic auditory stimulus control was achieved, ED was 0 before, during, and after the session. The sound was then presented during ED to determine the effects of ED on response accuracy, which declined from 100% to chance levels. When discriminative stimuli were presented during ED, ED increased before, during and after sessions. Conclusions: ED can be suppressed by operant processes, including reinforcement and stimulus control. When frequent reinforcement is presented for operant behavior in the absence of ED, ED decreases, especially after discrimination training, and this effect persists in the training context. Similar results were obtained in all three subjects. Concurrent EEG and behavioral intervention offers a mature, useful and effective non-pharmacological intervention to substantially suppress seizures.

Disclosures: J.C. Neill: None. **N. Alvarez:** None.

Poster

535. Epilepsy: Human Studies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.20/W4

Topic: C.08. Epilepsy

Support: The Patrick and Catherine Weldon Donaghue Medical Research Foundation

Betsy and Jonathan Blattmachr family

Title: Prospective testing of driving during clinical and subclinical seizures in patients with epilepsy

Authors: *W. CHEN[1], A. BAUERSCHMIDT[1], M. W. YOUNGBLOOD[1], C. CUNNINGHAM[2], C. EZEANI[2], Z. KRATOCHVIL[2], J. BRONEN[2], J. THOMSON[2], K. RIORDAN[2], J. Y. YOO[2], R. SHIRKA[2], L. MANGANAS[2], L. J. HIRSCH[2], H. BLUMENFELD[2];
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Abstract: Epileptic seizures, especially when associated with loss of consciousness, can significantly limit the ability of affected individuals to lead normal, independent lives. Because seizures are often associated with loss of motor control and loss of consciousness, people with epilepsy (PWE) are restricted from driving until it can be shown that seizures are fully controlled. In the U.S. and elsewhere, driving license issuance is dependent on PWE maintaining a seizure-free period whose length depends on local laws. The individual's physician plays an important role in determining whether or not the patient should be allowed to drive. However, these decisions are difficult because little objective data are available about patient driving ability during seizures. In this study we analyzed ictal and interictal performance data captured prospectively from a driving simulator in patients undergoing continuous video/EEG monitoring. A total of 33 seizures in 20 patients were analyzed. Driving performance data during interictal periods were used as baselines in comparison to ictal driving performance. A set of quantitative criteria were used to determine the presence of impairment. These included car velocity, steering wheel movement, and application of the brake pedal, as well as whether crashes occurred. We found that seizures were associated with a higher rate of crashes than driving on the same portions of the track in the interictal period . In addition, longer duration of partial seizures was related to more severe impairment in driving. Subclinical electrographic seizures were not associated with obvious driving impairment. Ongoing analyses will determine if more subtle impairments occur in subclinical seizures. These findings demonstrate the feasibility of testing

ictal driving in a prospective manner. In future work we hope to determine whether specific seizure types or localizations present a greater driving risk, with the goal of providing improved guidance to physicians and patients with epilepsy.

Disclosures: W. Chen: A. Employment/Salary (full or part-time):; Yale University. **A. Bauerschmidt:** None. **M.W. Youngblood:** None. **C. Cunningham:** None. **C. Ezeani:** None. **Z. Kratochvil:** None. **J. Bronen:** None. **J. Thomson:** None. **K. Riordan:** None. **J.Y. Yoo:** None. **R. Shirka:** None. **L. Manganas:** None. **L.J. Hirsch:** None. **H. Blumenfeld:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.21/W5

Topic: C.08. Epilepsy

Support: Grant-in-Aid for Young Scientists (13274571), JSPS

Grants-in-Aid for Scientific Research (13369989), MEXT

Grant (24-7) for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare, Japan

Title: Characteristic neuronal activities of patients with mesial temporal lobe epilepsy: an *In vitro* imaging study of the resected non-sclerotic hippocampus

Authors: *H. KITAURA[1], H. MASUDA[2], H. SHIMIZU[3], H. SHIROZU[2], H. MURAKAMI[2], H. TAKAHASHI[3], S. KAMEYAMA[2], A. KAKITA[3];
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Abstract: Mesial temporal lobe epilepsy (MTLE) is the most frequent focal epileptic syndrome, and the vast majority of seizures originate from the mesial structures of the temporal lobe. Tissues surgically resected from the hippocampus usually exhibit severe neuronal loss, i.e. hippocampal sclerosis. Recently, however, we had the opportunity of examining histopathologically rare examples of hippocampus tissue taken from four patients with MTLE, where neuronal loss was not apparent. A brain MRI study of each patient demonstrated a tumor-like lesion in the uncus, a cavernous angioma in the uncus, slight atrophy of the unilateral hippocampus, and suspicious structural variation in the hippocampus, respectively. After surgical resection of the mesial temporal lobe, we investigated neuronal

activity in freshly prepared slices of the relatively well preserved hippocampi employing flavoprotein fluorescence imaging *in vitro*. As a control, we retrieved two non-MTLE patients with cortical tubers or a scar lesion in the lateral temporal lobe, in whom the surgically resected hippocampi showed no histopathologic abnormalities. Morphometric analysis disclosed no significant differences in the number or size of the hippocampal pyramidal neurons between the MTLE and control patients. However, the neuronal activities detected in the slices differed. Although flavoprotein fluorescence responses were observed in all subfields in all patients, the amplitudes of the responses in the subiculum and CA1 were significantly larger in the MTLE patients than in the controls (mean ± SD (%); MTLE vs. control = 2.01 ± 0.32 vs. 0.79 ± 0.37, respectively in the subiculum, and 2.07 ± 0.83 vs. 0.83 ± 0.29, respectively in CA1). Local field potential recording demonstrated epileptiform spontaneous discharges in the subiculum and CA1 only in the MTLE patients. Moreover, the epileptiform activities had a clear high-frequency oscillation component, and surprisingly the discharge in the subiculum always preceded that in the CA1 by about 50 ms. Currently it is unclear whether the characteristic neuronal activities were specific for the MTLE patients we examined, or are universal in patients with the early stage of common MTLE. Further studies are needed to clarify the epileptogenic mechanisms in MTLE patients with hippocampal sclerosis. The present results suggest that in MTLE patients with no hippocampal sclerosis, epileptogenesis may occur in the subiculum.

Disclosures: H. Kitaura: None. **H. Masuda:** None. **H. Shimizu:** None. **H. Shirozu:** None. **H. Murakami:** None. **H. Takahashi:** None. **S. Kameyama:** None. **A. Kakita:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.22/W6

Topic: C.08. Epilepsy

Support: ICyT PFUT-08-027

FIS/IMSS/PROT/737

103937 CONACYT

Title: Study of polymorphisms in the coding regions of cyp2d6 and cyp2c19 genes associated with metabolism of antiepileptic drugs in mexican childrens with refractary epilepsy

Authors: *M. A. LÓPEZ GARCÍA[1], S. OROZCO-SUAREZ[2], I. FERIA ROMERO[2], H. FERNANDO SERRANO[3], I. GRIJALVA OTERO[4], D. RAYO[5], M. FRAIRE[5], I. VERGARA[5],

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Abstract:
Antiepileptic drugs are metabolized by enzymatic reactions of the family of cytochrome P450(CYP). Genetic variants lleles) of CYP affect plasma concentrations or antiepileptic drug exposure. CYP2D6 and CYP2C19 are polymorphic genes associated with metabolism that have been associated with underlying risks. The purpose of this work is to relate the concentration of antiepileptic drugs in blood and saliva metabolized by CYP450 polymorphisms in genes encoding these enzymes. We included 30 patients, 23 drug resistant and 7 drug-controlled, with an average age of 10 years and a predominance of women (60%) in both groups; 50% began at 3 years of age and 75% of the drug-presented their first crisis within their first nine years of life, one more than the controls. The treatment usedwas valproic acid; average dose ingested and 025.4mg/24h 1140mg/24h respectively. The saliva concentration was 2.3mg in the control group and 1.0 mg in the problem group; the concentration in blood plasma was of 82.1mg and 63.2mg respectively. The average consumption of valproic acid in 24h in the control group varied evenly between the minimum dose and maximum consumed (42mg). In the drug group, this difference increased to 620mg. However, according to the genetic variants, generally valproic acid concentrations in the saliva howed higher concentrations in the control group than in the refractory (average of 2.4 and 1.0 mg, respectively), and with respect to the average plasma it was 83.9mg in the control group and 65.1mg in the problem group. Frequencies of genetic variants of the SNP's tracked exons 1, 3, 5 and 6 of the CYP2D6 gene 4 and 5 of CYP2C19, showed an increased
prevalence in heterozygotes of all exons of the control group except in exon 5 CYP2D6 gene, likewise the refractory group showed heterogeneity in the prevalence of the variant studied exons. And for the gene CYP2C19, polymorphisms were identified only in heterosigota form, as expected. So far no relationship has been found between the SNP's and the drug concentration in plasma and saliva. The polymorphisms of CYP450 genes can contribute to an individualized therapy in epilepsy treatment.(Work supported by ICyT PFUT-08-027,FIS/IMSS/PROT/737 and fellow 103937 CONACYT).

Disclosures: M.A. López García: None. **S. Orozco-Suarez:** None. **I. Feria Romero:** None. **H. Fernando Serrano:** None. **I. Grijalva Otero:** None. **D. Rayo:** None. **M. Fraire:** None. **I. Vergara:** None. **P. Fagiolino:** None.

Poster

535. Epilepsy: Human Studies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.23/W7

Topic: C.08. Epilepsy

Support: NIH Grant NS49306

NIH Grant NS64154

Title: Genomic variation associated with common forms of human epilepsy

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Abstract: We previously reported identification of putative epilepsy susceptibility loci using data from a genome wide association study (GWAS). Since our initial data presentation, we genotyped additional samples and have thereby increased power to detect single nucleotide polymorphism (SNP) association from individuals of European (n=1364) and African (n=271) ancestry, all of whom passed rigorous quality control measures and are included in analyses reported here. Epilepsy subtypes included genetic generalized epilepsy (GGE: n=656 European, n=114 African) and non-symptomatic/cryptogenic focal epilepsy (CFE: n=708 European, n=157 African). DNA from healthy control individuals was used for comparison (European n = 6419, African n=2843). Samples were genotyped on the Illumina 550,610, and Omniexpress chips and 38 million variants were imputed with the 1000 genomes project haplotypes. Single-point contingency analysis was carried out using the European samples alone or in combination with samples of African ancestry comparing all patients with all controls, all CFE patients to all controls and all GGE patients to all controls. In a comparison between European GGE and matched controls, six linked markers in the PADI6 locus on chr1p36 reached p values suggestive of genetic association ($p<10^{-7}$) and the top marker reached genome wide significance (rs34018214, $p=4.97\times10^{-8}$). Several additional loci demonstrate suggestive association with GGE including markers on chr1 (CNIH3), chr4 (GRID2), chr6 (SLC35F1) and chr16 (XYLT1). Indeed, our prior data identified association at the MHY11/NDE1 gene locus at 16p13.11, a region within 2MB of XYLT1 and where deletions have been associated with seizures, intellectual disability, autism, and schizophrenia. Suggestive evidence for an association with

focal epilepsy was found on chr2 (RTN4), chr7 (SP8), chr9 (ZNF462), and chr19 (NUDT19). When GGE and CFE patients were combined, suggestive evidence for an association was identified on chr2 (GLS, NAB1) and chr20 (PTPRT). Copy number variation (CNV) analysis on this cohort is in progress and GWAS data sets from around the world are being combined for a meta-analysis that will include approximately 10,000 patients and 20,000 controls. It is anticipated that increased statistical power afforded by meta-analysis will confirm identified suggestive loci as bona fide epilepsy susceptibility genes. These new data confirm some earlier findings and identify new gene targets for further investigation in the pathophysiology of common forms of human epilepsy.

Disclosures: R.J. Buono: None. **J. Bradfield:** None. **Z. Wei:** None. **M.R. Sperling:** None. **D. Dlugos:** None. **W. Lo:** None. **T.N. Ferraro:** None. **H. hakonarson:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.24/W8

Topic: C.08. Epilepsy

Support: donativos ICy T del GDF,(PIFUTP08-027)

Fondo de Investigación en Salud (FIS/ IMSS/PROT/737)

Title: ABCB1 gene polymorphisms in Mexican pediatric patients with drug-resistant complex partial epilepsy

Authors: *D. ESCALANTE SANTIAGO[1], S. OROZCO-SUAREZ[2], I. FERIA-ROMERO[5], D. RAYO-MARES[3], R. RIBAS-APARICIO[6], I. GRIJALVA-OTERO[4], P. FAGIOLINO[7]; [1]Inst. Mexicano Del Seguro Social, Chalco. Edo De Mexico, Mexico; [2]Hosp. de Especialidades. Ctr. Médico Nacional Siglo XXI, [3]Hosp. de Pediatría. Ctr. Médico Nacional Siglo XXI, [4]Hosp. de Especialidades, Inst. Mexicano del Seguro Social, Mexico D.F, Mexico; [5]Hosp. de Especialidades. Ctr. Medico Nacional S. XXI, Inst. Mexicano del seguro Social, Mexico D.F, Mexico; [6]Escuela Nacional de Ciencias Biológicas, Inst. Politecnico Nacional, Mexico D.F, Mexico; [7]Ciencias Farmaceuticas, Univ. de la Republica, Montevideo, Uruguay

Abstract: One factor associated with drug resistance in epilepsy is genomic variability, as the presence of polymorphisms that produce alterations in the protein and P-glycoprotein (Pgp) encoded by the ABCB1 gene, which acts as an efflux pump in the blood brain barrier and controlling limiting entry drugs and toxins into the brain, various substrate antiepileptic drugs

(AEDs) like felmabato, gabapentin, lamotrigine, topiramate and phenytoin. Alterations are polymorphic; it may be generating alterations in the adsorption of AEDs.
The aim of this work was to identify the polymorphisms rs9282564, rs2229109, rs1128503, rs28381209, rs2032582, rs1045642 and rs28364274 ABCB1 gene in drug-resistant patients with TLE and its relationship to plasma levels and salivary DAEs.
Of the polymorphisms analyzed, 4 are present in Mexican pediatric population (rs2229109, rs1128503, rs2032582 and rs1045642). The SNPs C1236T was the most frequent (59.1%), with 61.5% homozygosity. This polymorphism is synonymous. The T2677G SNPs that changes a serine to alanine, was presented in 54.5% of the study population, with a ratio of 50% of heterozygotes and homozygotes. The third most frequently was polymorphism C3435T (48.6%), with a trend homozygous allele (53.8%). The G1199A SNPs was less frequent (9.09%). T2677G polymorphism had higher relationship with AEDs. However, no significant association was found between SNPs and drug ELT. However, in our study population was a tendency to present the C1236T polymorphism.
It is necessary to perform a study with a larger number of patients with this pathology with the most significant polymorphisms found in this study to identify the involvement of Pgp in drug-resistant epilepsy.

Disclosures: D. Escalante Santiago: None. **S. Orozco-Suarez:** None. **I. Feria-Romero:** None. **D. Rayo-Mares:** None. **R. Ribas-Aparicio:** None. **I. Grijalva-Otero:** None. **P. Fagiolino:** None.

Poster

535. **Epilepsy: Human Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 535.25/W9

Topic: C.08. Epilepsy

Support: National Institute of Neurological Disorders and Stroke, Contract # HHSN271201100029C.

Title: A novel approach to model the consequences of non-adherence in newly diagnosed patients with epilepsy

Authors: *K. E. THOMSON[1], C. RUEDA[2], A. C. MODI[3], T. A. GLAUSER[4], S. WHITE[2]; [1]Anticonvulsant Drug Develop. Program, SALT LAKE CTY, UT; [2]Pharmacol. and Toxicology, Univ. of Utah, SALT LAKE CTY, UT; [3]Div. of Behavioral Med. and Clin. Psychology, [4]Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Epilepsy is a debilitating seizure disorder that can profoundly impact quality of life. It is estimated that 38% of patients with epilepsy do not have adequate seizure control with their current anti-seizure drug (ASD) regimen. This percentage has remained relatively constant over the past 20 years, despite 20 new ASD compounds made available. However, it is not known whether non-adherence to the ASD regimen directly contributes to poor seizure control and the development of therapy resistance. Clinical results have suggested that early non-adherence is associated with differential long term seizure control. However, these results are confounded by many factors, including inaccurate self-reporting of seizures, patient overestimation of adherence, and the various etiologies and severities of human epilepsy. As such, it is difficult to directly evaluate the effects of non-adherence on the occurrence of seizures. There is evidence in the field of epilepsy research that quick withdrawal of an ASD medication leads to the precipitation of seizures, that seizures lead to more seizures, and that a higher seizure burden leads to therapy resistant epilepsy. Taken together, these three pieces of evidence suggest the hypothesis that non-adherence behaviors may be detrimental to seizure control. Given the problems associated with testing this hypothesis in the clinical setting, we have developed a unique approach using an established animal model to test the impact of non-adherence on seizure control.
We have designed a novel computer-automated medication-dosing system using a carbamazepine(CBZ)-in-food protocol (75 mg/kg p.o., QID). An excess number of Sprague Dawley rats were implanted with EEG electrodes and treated with low-dose kainate to permit a minimum of 8 epileptic rats enrolled into each experimental arm. After their first observed spontaneous convulsive (Racine Stage 3+) seizure, the animal was randomly enrolled into one of three treatment groups; 100% medication, no medication, or a randomized pattern of 50% adherence for the remaining 6 weeks of the study. This is the first study to use an animal model to study the impact of human non-adherence patterns on seizure control. Our preliminary results suggest that non-adherence to the prescribed ASD regimen employed in this study may contribute to poor seizure control. The development of a computer controlled automated feeder system and the use of newly diagnosed epileptic rats into a clinical protocol designed to mimic the management of newly diagnosed epilepsy will provide new insight into the impact of non-adherence on seizure control and development of therapy resistance.

Disclosures: K.E. Thomson: None. **S. White:** A. Employment/Salary (full or part-time):; NeuroAdjuvants. **C. Rueda:** None. **T.A. Glauser:** None. **A.C. Modi:** None.**Poster**

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.01/W10

Topic: C.08. Epilepsy

Support: NIH Grant R01-NS074450

Title: Rats with epilepsy reinforce poor spatial information during sleep following a spatial task

Authors: *A. S. TITIZ, M. MAHONEY, M. TESTORF, P.-P. LENCK-SANTINI, G. HOLMES, R. SCOTT;
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Abstract: Memory impairment is a major problem in patients with temporal lobe epilepsy (TLE) which is accompanied by disturbances in sleep. As sleep is critical for memory consolidation, we hypothesized that rats with TLE would have impairments in the sleep reactivation of neuronal patterns observed during behavior. In this study, we recorded place cells while animals were running in a circular track and then evaluated the reactivation of the neuronal patterns observed in the following sleep period. Place cell coherence was decreased in animals with TLE ($p < 0.05$). Despite poor quality place cells, we show evidence for normal neuronal reactivation in epileptic rats using a correlation approach. We then examined the sequential activation of place cells during population bursts in sleep and found that in both groups of animals these bursts are represented by high-order Markov Chains (i.e., four- and five-grams) similar to the sequences observed in behavior. Thus, the reactivation system appears to remain intact in animals with TLE despite severe cell loss and recurrent epileptiform activity. Therefore, sleep related memory processing in the hippocampus may be intact in the pilocarpine model of TLE. We propose that the memory impairments TLE may be at least in part a result of consolidation of poor spatial information encoded in the hippocampus during behavior.

Disclosures: A.S. Titiz: None. **M. Mahoney:** None. **M. Testorf:** None. **P. Lenck-Santini:** None. **G. Holmes:** None. **R. Scott:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.02/W11

Topic: C.08. Epilepsy

Support: NIH Grant NS31348

Title: Genetic modifiers of generalized epilepsy mutations in mice

Authors: *W. N. FRANKEL, T. C. MCGARR, C. L. MAHAFFEY, B. J. BEYER, V. A. LETTS;
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Abstract: Epilepsy is a genetically complex disease. Even so-called Mendelian gene effects can be influenced profoundly by other genetic variants in an individual's genome. Identifying and understanding how natural variants modify disease should provide insight towards treatments or cures. Unbiased approaches such as forward genetics increase the chance that these discoveries will be novel or unexpected.
We have studied mutant genes that cause absence seizures in mice, including *Gria4* (AMPA receptor subunit 4), *Scn8a* (sodium channel $Na_v1.6$) and *Gabrg2* (GABA-A receptor subunit gamma 2). Genetic modifiers are clearly at play in each: on the C57BL/6J (B6J) strain background compared with C3HeB/FeJ (FeJ) each presents a significantly mitigated phenotype in the EEG - fewer spike-wave discharges (SWD), shorter average duration. To investigate whether these modifiers are shared or private, we performed genetic mapping and validated several chromosomal regions. *Gabrg2* and *Gria4* mutations appear to share a major modifier on Chr 6 (in the process of being confirmed). *Gria4* has a major "private" modifier on Chr 15 (confirmed in a congenic strain). *Scn8a* does not appear to share a modifier with the other two mutations, but has its own private modifier on Chr 7 (also confirmed). We recently completed RNAseq gene expression from thalamus and cortex in B6J and FeJ and have begun testing both novel and known candidate genes within mapped modifier regions.
Beyond major strain differences, even mouse substrains may show modifier effects. SWD due by *Gria4* mutation are more mitigated on the C3H substrain HeJ compared with FeJ – these are very closely related except for recent genetic drift; in C3H strains a major cause of *de novo* events are IAP retrotransposon insertion mutations. We identified a HeJ-specific insertion in a gene that encodes a conserved, novel transmembrane protein, and exploited TALEN technology to create a new frameshift mutation in FeJ. Our preliminary results suggest that this gene is responsible for at least some of the substrain difference. We are confirming and examining this gene in greater detail and determining whether it also affects the phenotype of other generalized epilepsy mutations. We hope that this and other novel or unexpected discoveries will add to the armament of new tools to understand and treat seizure disorders.

Disclosures: W.N. Frankel: None. **T.C. McGarr:** None. **C.L. Mahaffey:** None. **B.J. Beyer:** None. **V.A. Letts:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.03/W12

Topic: C.08. Epilepsy

Support: ICMR, New Delhi (45/33/2010/PHA-BMS)

Title: Psychoneurochemical investigations to reveal neurobiology of depression, learning and memory deficit in epilepsy

Authors: *R. K. GOEL[1], A. MISHRA[2];
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Abstract: Exploration of neurochemical changes in discrete brain parts linked to epileptogenesis and memory formation may help to understand the neurobiology of susceptibility to depression, learning and memory deficit in epileptic patients and progressive learning and memory deficit in uncontrolled or untreated patients with epilepsy. In chronic epileptic patients, ictal and postictal states are relative momentary than interictal state, which persists for longer time. As the time span of the interictal state prevails over postictal state, the monitoring of behavioral and neurochemical changes in the interictal state may be crucial to provide valuable insight for the pathology of susceptibility of learning and memory deficit in epilepsy. While for understanding the neurobiology of progressive depression, learning and memory deficit in uncontrolled seizures neurochemical changes may be monitored after multiple convulsive episodes. Therefore the present study was executed to explore the possible targets for comprehensive management of this problem.

Kindling was induced by administering subconvulsive dose of pentylenetetrazole (35 mg/kg; i.p.) at an interval of 48±2 h. Only successfully kindled animals were included in the study and divided into two groups (interictal and postictal group), while non-kindled animals served as naïve group. In postictal group, animals were challenged with pentylenetetrazole (35 mg/kg) on days 5, 10, 15 and 20. Depression and learning and memory were evaluated on days 5, 10, 15 and 20. After behavioral evaluations on day 20, all animals were sacrificed to remove their brains for estimation of glutamate, GABA, norepinephrine, dopamine, serotonin, acetylcholinesterase activity and total nitrite.

The results of the study suggest that pentylenetetrazole-kindling in mice associated depression like behavior, learning and memory deficit in interictal group and progressive memory deficit in postictal group. Neurochemical changes suggest, reduction in monoamines level and elevation in nitrosative stress and acetylcholinesterase activity in hippocampus and cortex as a possible pathogenic mechanism, apart from glutamate/GABAergic hypothesis, for the development of depression, learning and memory impairment in chronic model of experimental epilepsy. Further, selective enhancement hippocampal and cortical monoamines level may be useful as comprehensive target and reduction in nitrosative stress and acetylcholinesterase activity in hippocampus and cortex may be useful as adjuvant targets with antiepileptic drugs for the management of depression, learning and memory deficit in epilepsy.

Disclosures: R.K. Goel: None. **A. Mishra:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.04/W13

Topic: C.08. Epilepsy

Support: Mackpesquisa

CNPq

CAPES

Title: Early life seizures in female rats lead to anxiety-related and abnormal social behaviors

Authors: *R. M. CYSNEIROS, A. S. S. CASTELHANO, G. S. T. CASSANE;
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Abstract: Neonatal seizures are the most common manifestation of neurological dysfunction in the neonate. Animal data indicates that seizures during development are associated with a high probability of long-term adverse effects such as learning and memory impairment and behavioral changes. Previously, we reported that a single episode of neonatal status epilepticus (SE) impaired social play behavior in male rats (Castelhano et al., 2010), increased emotionality and impaired sociability with no locomotor activity changes (Castelhano et al., 2013, in press). We evaluated the sociability and anxiety-like behaviour of female rats submitted to neonatal SE. Experimental group (EXP) received pilocarpine (380 mg/kg, i.p) and control group (CTR) received saline at postnatal day 9. Behavioral studies started at 60 days postnatal. To the time of immobility, we noted a significant effect of group ($F[1.22]= 23.41$; $p< 0.0001$) and of the exposure in the open field´s arena (1st X 2nd, $F[1.22]= 45.21$; $p< 0.0001$), and no effect of interaction ($F[1.22]= 3.34$, NS). EXP exhibited higher time of immobility comparatively to CTR in both exposures with reduced central locomotor activity ($F[1.22]= 24.18$; $p<0.0001$). However, the total locomotion did not differ between groups ($F[1,22]= 0.57$; NS), suggesting the presence of increased emotionality with no alterations in general locomotor activity. The sociability was investigated measuring the tendency of the rat to approach an unfamiliar rat and engage in social investigation. With the introduction of an unfamiliar rat into one of the compartments, EXP exhibited less number of entries as compared to the CTR into the compartments ($F[1.22]= 45.90$, $p <0.0001$), but not between compartments ($F[1.22]= 0.53$; NS), and a significant effect of interaction was noted for time spent into compartments ($F[1.22]= 12.58$; $p=0.0018$). CTR spent

more time with unfamiliar rat than with object and EXP did not show interest for social encounter. With an introduction of a novel rat into another wire cage, EXP exhibited fewer number of entries into compartments ($F[1.22]=92.45; p<0.0001$), but not between compartments ($F[1.22]=3.61$; NS and). For time spent into compartments, a significant effect of interaction was noted ($F[1.22]= 39.66$; $p<0.0001$). CTR spent more time with a novel rat and EXP did not exhibit motivation for social novelty. For snout-snout contacts a significant effect of interaction was noted ($F [1.22]= 79.11$, $p<0.0001$). CTR exhibited more preference for the social novelty than EXP. The data suggest that neonatal SE in rodents leads to altered anxiety-related and abnormal social behaviors.

Disclosures: R.M. Cysneiros: None. **A.S.S. Castelhano:** None. **G.S.T. Cassane:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.05/W14

Topic: C.08. Epilepsy

Support: NIH Grant R01NS073083

NIH Grant R01NS075249

NIH Grant R01NS076763

Emmory R. Shapses Research Fund

Great Ormond Street Children's Charity

Investigator-Initiated Grant Questcor Pharmaceuticals

Title: Focal epileptiform activity in the prefrontal cortex is associated with long-term attention and sociablity deficits

Authors: *A. E. HERNAN[1], A. ALEXANDER[1], K. JENKS[1], J. BARRY[1], P.-P. J. LENCK-SANTINI[1], E. ISAEVA[1,2], G. L. HOLMES[1], R. C. SCOTT[1,3];
[1]Neurol., Geisel Sch. of Med. at Dartmouth, Lebanon, NH; [2]State Key Lab. for Mol. and Cell. Biol., Kiev, Ukraine; [3]Inst. of Child Hlth., Univ. Col. London, London, United Kingdom

Abstract: There is a well-described association between childhood epilepsy and pervasive cognitive and behavioral deficits including working memory impairments, ADHD and autism

spectrum disorder. However, the precise role of epileptiform discharges in these deficits remains unclear. In order to understand the relationship between frequent epileptic discharges during the neurodevelopmental period and cognition later in life, we developed a model of frequent focal interictal spikes (IIS). Postnatal day (p) 21 rat pups received intracortical injections of bicuculline methiodine into the prefrontal cortex (PFC) while EEG was continuously recorded. Within seconds of injection, focal spikes were recorded at the injection site. Injections were repeated in order to achieve 5 days of IIS. Short-term plasticity (STP) and behavioral outcomes were studied. IIS resulted in a significant increase in STP bilaterally in the PFC. In a delayed non-match-to-sample task IIS rats showed marked inattentiveness without deficits in working memory. Rats also demonstrated deficits in sociability. No changes in motivation, hyperactivity or anxiety were seen. We conclude that early-life focal IIS in the PFC have long-term consequences for cognition and behavior at a time when IIS are no longer present. Focal IIS during development can disrupt neural networks, lead to long-term deficits and thus may have important implications in attention deficit disorder and autism.

Disclosures: **A.E. Hernan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Questcor Pharmaceuticals. **A. Alexander:** None. **K. Jenks:** None. **J. Barry:** None. **P.J. Lenck-Santini:** None. **E. Isaeva:** None. **G.L. Holmes:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Questcor Pharmaceuticals. **R.C. Scott:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.06/W15

Topic: C.08. Epilepsy

Support: NIH Grant DC012425

Title: Mossy fiber sprouting in the BDNF-enriched hippocampus

Authors: *K. M. GUTHRIE, C. AYDIN, C. PARE, C. ISGOR;
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Abstract: Structural changes that alter hippocampal functional circuitry are implicated in learning impairments, epilepsy, and mood disorders. Sprouting of mossy fiber (MF) axon collaterals from dentate gyrus granule cells (GCs) is one such form of plasticity that results in expanded innervation of the supragranular layer and CA3 pyramidal neurons. Hippocampal levels of the neurotrophin brain-derived neurotrophic factor (BDNF) are regulated by activity,

and seizures increase BDNF expression, as well as TrkB receptor activation, in the MF-CA3 projection. Seizure activity also promotes MF sprouting, implicating BDNF signaling in this structural response. In transgenic mice with reduced BDNF levels/TrkB signaling, seizure induction and MF sprouting are suppressed. Mice overexpressing BDNF under the beta-actin promoter show increased seizure susceptibility, and enhanced GC dendritic complexity, but surprisingly, no MF sprouting out to 6 months of age. Mice that overexpress BDNF under the alpha-calcium/calmodulin-dependent protein kinase IIa promoter (termed TgBDNF mice) show an age-dependent emergence of epileptogenesis, with spontaneous seizures developing at ~5 months. Seizures are not observed earlier, but cognitive effects are evident by 2-3 months and include impairments in spatial memory. This suggests early circuit disruptions that may progress under the influence of chronically elevated BDNF to promote epileptogenesis. To determine if young adult TgBDNF mice show structural changes in hippocampus, we used Timm's method for silver sulfide staining and quantitative stereology to measure MF terminal field volumes in 8-week-old TgBDNF and wild-type mice. In situ hybridization and Western blotting were used to compare levels of hippocampal BDNF and TrkB-mediated signaling. TgBDNF mice showed a ~5-fold increase in mature BDNF, with no significant changes in steady-state levels of TrkB, ERK1/2, and PLC-gamma-1 phosphorylation. In contrast to beta-actin-BDNF mice, TgBDNF mice exhibited significant MF sprouting, as evidenced by 30% and 50% increases in terminal field volumes in the suprapyramidal and intra/infrapyramidal compartments, respectively. These data support a role for sustained increases in BDNF in the development of aberrant MF-CA3 functional connectivity.

Disclosures: K.M. Guthrie: None. **C. Aydin:** None. **C. Pare:** None. **C. Isgor:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.07/W16

Topic: C.08. Epilepsy

Support: NIH Grant NS058585

Epilepsy Foundation of America Predoctoral Fellowship

University of Michigan Center for Organogenesis Predoctoral Fellowship

Title: Electrophysiological properties of age-defined dentate granule cells in a rodent model of temporal lobe epilepsy

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Abstract: Dysregulated hippocampal neurogenesis is a prominent feature of temporal lobe epilepsy (TLE). Anatomical data indicate that most dentate granule cells (DGCs) generated in response to an epileptic insult develop features that promote increased excitability, including ectopic location, persistent hilar basal dendrites (HBDs) and mossy fiber sprouting. However, some appear to integrate normally and promote reduced excitability. Much of what is known about aberrant DGC neurogenesis comes from anatomical data; relatively few studies have investigated the physiological properties of age-defined cells. Using a retroviral (RV) GFP reporter to birthdate DGCs, our laboratory found that DGCs that were mature at status epilepticus (SE – the onset of epilepsy in this model) are resistant to morphological abnormalities, while the majority of those born after SE display TLE-related pathology. This may suggest that post-SE generated DGCs promote pathological function while established DGCs retain normal function. To examine the relationship between DGC age and activity within an epileptic network, we record from RV birth-dated DGCs born either neonatally, or during adulthood in an epileptic or intact animal. Recordings are made between 8-14 weeks after SE from age-defined cells in acute hippocampal slices using voltage-clamp. We find that, in TLE tissues, both adult-born and neonatal-born populations of DGCs receive increased excitatory input compared with age-matched controls in intact tissues. Ongoing analysis aims to determine whether there is a correlation between degree of aberrant morphology and amount of excitatory input. Concurrent experiments will assess inhibitory inputs within the same populations of cells. A separate set of experiments confirmed that intrinsic properties that influence excitability such as input resistance, membrane potential and action potential threshold are not different among groups.

Disclosures: A.L. Althaus: None. **G.G. Murphy:** None. **J.M. Parent:** None. **H. Zhang:** None. **E. Messenger:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.08/W17

Topic: C.08. Epilepsy

Support: Department of Defense (USAMRMC) Grant W81XWH-11-1-0502

American Epilepsy Society and Lennox & Lombroso Trust for Epilepsy Research and Training Fellowship

Title: Inhibitory signaling to dentate granule cells following traumatic brain injury

Authors: *C. R. BUTLER[1], J. A. BOYCHUK[1], B. N. SMITH[1,2];
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Abstract: Traumatic brain injury (TBI) is one of the most common causes of temporal lobe epilepsy (TLE). Changes in inhibitory signaling after CCI include hilar inhibitory neuron loss, synaptic reorganization, and GABA receptor subunit reorganization, all of which may support the development of spontaneous seizures following TBI. Here, a mouse model of posttraumatic epilepsy using controlled cortical impact (CCI; velocity= 3.5m/s; duration= 500 msec; depth= 1.0mm) was used to examine inhibitory signaling within the dentate gyrus 8-13 weeks after injury. Tonic and phasic GABAA receptor-mediated responses were assessed using whole cell patch-clamp recordings of dentate granule cells (DGCs) in slices from control and injured mice. Both tonic and phasic GABAA responses were studied in voltage clamp at baseline and during steady-state bath application of 4,5,6,7-tetrahydroisoxa-zolo[5,40c]pyridin-3-ol (THIP) (3 μM) followed by bicuculline (30 μM) to explore δ subunit containing GABAA receptor function in DGCs after TBI.
Preliminary results indicate no significant difference in the total basal tonic current uncovered with bicuculline application in DGCs between CCI-injured and sham-operated control mice, or between ipsilateral and contralateral hippocampi of injured mice. However, the tonic current in DGCs from the injured hemisphere of CCI mice exhibited reduced sensitivity to THIP, suggesting a decreased contribution of δ subunit-containing GABAA receptors to tonic current generation. No significant differences between CCI-injured mice and sham-operated controls were detected in spontaneous inhibitory postsynaptic current (sIPSC) amplitude or kinetics, either at baseline or during THIP application. Results suggest that tonic GABAA receptor-mediated currents are altered ipsilateral to the injury after CCI in mice, a response that involves reduced activity of δ subunit-containing GABAA receptors several weeks after injury. Ongoing studies will assess inhibitory signaling in DGCs at earlier time points after injury.

Disclosures: C.R. Butler: None. **J.A. Boychuk:** None. **B.N. Smith:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.09/W18

Topic: C.08. Epilepsy

Support: NSF CAREER Award 1149446

Title: Aberrant hippocampal neuron organization in the seizure-prone naked mole-rat

Authors: M. ZIONS[1], X. A. GEOFFROY[2], C. VICIDOMINI[3], *D. P. MCCLOSKEY[3,1,2];
[1]Doctoral Program in Neurosci., The Grad. Ctr. at CUNY, New York, NY; [2]Ctr. for Developmental Neurosci., Col. of Staten Island/CUNY, Staten Island, NY; [3]Dept of Psychology and Program in Developmental Neurosci., Col. Staten Island/ CUNY, STATEN ISLAND, NY

Abstract: The unique ecological niche and social organization of naked mole-rats (Heterocephalus Glaber) make them a useful model to study environmental, hormonal, and social factors related to epilepsy. But perhaps the most relevant feature that makes them a useful animal model of epilepsy is their spontaneous seizure activity; naked mole-rats housed in a laboratory setting demonstrate motor seizure behaviors. In an effort to understand the mechanisms driving these seizures, the present work examined the hippocampus as a likely candidate structure. Electrophysiological and anatomical studies suggest altered hippocampal circuitry that may be related to the observed seizure behaviors.

Single channel and multielectrode array recordings of acute hippocampal slices of naked mole-rats demonstrate unprovoked multifocal epileptiform burst discharges in over 90% of recorded animals. The bursts that originate in area CA3 are markedly similar to those we have previously recorded in pilocarpine-treated chronic epileptic rats. Stimulation of the mossy fiber pathway reveals an antidromic signal from CA3 to DG which is absent under TTX. CNQX abolishes the orthodromic response but does not eliminate the antidromic signal. Di-I and biocytin labeling in the hilus confirm the presence of CA3 pyramidal cells with axons heading toward the dentate gyrus.

Initial multielectrode recordings demonstrate that additional bursts are generated in the subiculum, producing prolonged epileptiform discharges which appear to be independent of CA3. We are exploring the implications of this restructured hippocampal circuitry with graph theory-based network analysis of single neuron interactions across the hippocampal slice; we also are confirming broader seizure activity with in vivo recording from live naked mole-rats.

Disclosures: M. Zions: None. **X.A. Geoffroy:** None. **D.P. McCloskey:** None. **C. Vicidomini:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.10/X1

Topic: C.08. Epilepsy

Support: NS32403

NS38572

GM081259-05

1F31NS080403 - 01A1

Title: Development of corrupted dentate granule cell activation properties in a mouse model of temporal lobe epilepsy

Authors: *C. G. DENGLER[1], S. F. FRAUSTO[2], H. TAKANO[2], D. A. COULTER[1,2]; [1]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [2]Children's Hopsital of Philadelphia, Philadelphia, PA

Abstract: In addition to its contribution to cognitive function, the dentate gyrus also plays a critical role in regulating the activation of downstream structures in the hippocampus and limbic system, acting as a regulated gate restricting the relay of synchronous neuronal activity associated with epilepsy. Dentate granule cells (DGC) exhibit sparse, precise, and frequency-dependent network activation properties in vitro, which is consistent with in vivo observations. These properties of DGCs deteriorate in animal models of epilepsy. Here we investigated the time course of network activation properties of DGCs in the pilocarpine mouse model of temporal lobe epilepsy to determine if and how DGC activation is altered over the course of disease progression.

To accomplish this, we utilized multicellular calcium imaging to probe activation of DGCs responding to afferent stimulation in hippocampal entorhinal-cortex slices prepared from adult mice in a pilocarpine model of epilepsy. We recorded from naïve animals, animals early during epilepsy expression (2 weeks post status epilepticus, SE), and late-stage animals 6-12 months post SE. DGCs from both naïve animals and early stage animals exhibited sparse, frequency dependent activation of DGCs, with marked recruitment of DGCs at theta and gamma frequencies. However, DGCs from late-stage epileptic animals exhibited significantly higher proportional activation and the frequency dependence of this activation was degraded, with robust recruitment of DGC activity at all frequencies tested. Naïve DGCs exhibited significant precision in activation, which we defined as the probability that a given neuron would activate repeatedly given a prior history of activation. Eighty percent of naïve DGCs exhibited this precise firing pattern, compared to 60% of DGCs in late stage epilepsy, reflecting decreased firing precision. Additionally, we observed that as stimulus intensity increased, the amplitude of evoked calcium transients remained the same in DGCs from naïve animals. However, in late-

stage chronically epileptic animals, calcium transients were much larger, and much more variable in amplitude on average. Since calcium transient amplitude correlates with the action potential firing, DGCs in chronically epileptic mice are more likely to fire in bursts. Understanding how epilepsy alters the basic circuit properties of in hippocampal structures during the course of disease progression is important not only in targeting new therapies for seizure amelioration, but also in developing new treatments to reduce comorbidities accompanying epilepsy.

Disclosures: C.G. Dengler: None. **S.F. Frausto:** None. **H. Takano:** None. **D.A. Coulter:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.11/X2

Topic: C.08. Epilepsy

Support: FAPESP, CInAPCe-FAPESP, CNPq and FAPESP/CNPQ/MCT-Instituto Nacional de Neurociência Translacional.

Title: Hippocampal cell loss and moderate gliosis in rats injected with bethanechol

Authors: *J. C. DA SILVA[1], J. VALERO[3], J. O MALVA[4], E. ABRÃO CAVALHEIRO[2]; [1]Disciplina de Neurologia Exptl., [2]Laboratório de Neurologia Exptl. / Dpto de Neurologia e Neurocirurgia, Escola Paulista De Medicina/Universidade Federal De São Paulo - UNIFESP, São Paulo, Brazil; [3]Ctr. for Neurosci. and Cell Biology, Univ. of Coimbra, Coimbra, Portugal; [4]Fac. Med. Univ. of Coimbra, Coimbra, Portugal

Abstract: Purpose: Cholinomimetics are widely utilized to trigger status epilepticus (SE) and brain damage induced epileptogenesis. Intrahippocampal injection of bethanechol chloride (BeCh) produces sustained acute limbic seizures and widespread brain damage in rats. However, little is known about the chronic effects of this experimental protocol. Accordingly, this work aimed at observing long-term changes following intrahippocampal BeCh in rats. Methods: Adult male Wistar rats were housed under controlled conditions with food and water ad libitum (ethic committee of UNIFESP Nº 0157/11). Using stereotaxic surgery, 30 rats were implanted with electrodes for EEG recordings and with a guide cannula directed to the right hippocampus. Seven days after surgery, 20 experimental animals were intrahippocampally injected with BeCh (300µg/2µl saline), while 10 control animals received saline. Animals were then continuously video and EEG monitored for up to 180 days. After this period, the brains of three animals of

each group were removed, frozen and sectioned for neuropathological analysis. Results: In BECh treated animals acute behavioral and electrographic changes were similar to those previously reported by our group. The long-term study allowed observing that 9 out 20 rats presented spontaneous recurrent seizures, similar to those described in the pilocarpine model of epilepsy, after a latency period of 80±32 days. These behavioral seizures were accompanied in the EEG by an initial period of fast hippocampal theta (>12 Hz) followed by spikes that quickly spread to the neocortex. Neuronal death was observed in the in the hilus and in the dentate granule cell layer. In addition, reduction in the granule cell layer thickness and moderate gliosis in CA1 could also be observed in BeCh treated rats. Conclusion: Status epilepticus induced by intrahippocampal BeCh is able to induce, in the long run, epileptic behavioral, electrographic and anatomical changes similar to those described in the pilocarpine model of epilepsy. Interestingly, less than 50 % of BeCh treated animals evolved to the chronic epilepsy in contrast to 100% observed in the pilocarpine model. Further experiments are in progress aiming at explaining these differences.

Disclosures: J.C. Da Silva: None. **J. Valero:** None. **J. O Malva:** None. **E. Abrão Cavalheiro:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.12/X3

Topic: C.08. Epilepsy

Support: NIH Grant T32ES007051

NINDS R01NS065020

Cincinnati Children’s Research Foundation

Title: Mossy fiber axon abnormalities in a conditional PTEN knock out mouse model of temporal lobe epilepsy

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Abstract: Temporal lobe epilepsy is a serious neurological disorder in which spontaneous seizures are accompanied by neuroanatomical and physiological abnormalities in the hippocampus. Dentate granule cells, which are particularly vulnerable to developing structural

abnormalities, exhibit increased mammalian target of rapamycin (mTOR) signaling in status epilepticus models of epilepsy. Recently, our lab utilized Gli1-CreER^{T2} X PTEN$^{flox/flox}$ x GFP conditional knockout mice to demonstrate that selective deletion of the phosphatase and tensin homolog (PTEN) protein, an inhibitor of mTOR, from 10-25% of granule cells was sufficient to cause epilepsy. Spontaneous seizures began 6-8 weeks after gene removal in these animals (Pun et al., *Neuron*, 2012). Deletion of PTEN from granule cells resulted in somatic hypertrophy, mossy fiber sprouting, hilar basal dendrites, and ectopic somata. In the current study, we further investigated the morphological changes in the granule cell mossy fiber axons in these animals. Mossy fiber axons of dentate granule cells project through the hilus and into CA3, where they synapse onto apical dendrites of CA3 pyramidal cells via giant mossy fiber boutons. Mossy fiber axons in the stratum lucidum had a larger diameter in the PTEN knockout mice compared to controls, and stratum lucidum was thicker in the knockout mice, with axons sprouting into stratum oriens in some animals. Interestingly, the number of giant mossy fiber boutons per length of axon was reduced in the knockout mice compared to controls. Increased axonal thickness and sprouting could support improved communication efficiency between the dentate granule cells and CA3; however, the decrease in boutons per axon length may reflect a compensatory mechanism to suppress enhanced excitatory output. Further research is necessary to delineate the timing of these structural alterations in comparison to spontaneous seizures in this mouse model, as well as the net physiological effects from the collective morphological abnormalities.

Disclosures: C.L. LaSarge: None. **S.C. Danzer:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.13/X4

Topic: C.08. Epilepsy

Support: NIH Grant DA023675

Title: Dendritic analyses of hippocampal dentate gyrus granule and CA3 pyramidal neurons in brain-derived neurotrophic factor overexpressing mice

Authors: *C. ISGOR[1], F. HOSSAIN[1], C. AYDIN[2], O. OZTAN[1], K. GUTHRIE[1];
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Abstract: Mice that overexpress brain-derived neurotrophic factor (BDNF) under the alpha-calcium/calmodulin-dependent protein kinase IIa promoter (termed TgBDNF mice) show an age-

dependent emergence of epileptogenesis, with spontaneous seizures developing at ~5 months. Seizures are not observed earlier, but mild cognitive deficits are evident by 2-3 months and include impairments in spatial memory. This suggests early circuit disruptions that may progress under the influence of chronically elevated BDNF to promote epileptogenesis. We have previously shown that the mossy fibres, axonal projections from the dentate gyrus granule neurons that innervate the CA3 field, are expanded in volume in TgBDNF mice compared to wildtype (WT) controls at 2-3 months of age. Present study used Golgi-Cox staining technique to visualize the hippocampal neurons with their entire dendritic projections intact within thick sections (~200μm) using 2-3 month-old TgBDNF and WT mice. A computer-interfaced image analysis system (Neurolucida, Microbrighfield, VT) is used to reconstruct dendritic arbors to assess total dendritic length, number of dendritic branches, number of spines and spine density between TgBDNF and WT mice. Analyses of granule neuron reconstructions showed that TgBDNF mice had remarkably higher total dendritic length compared to WTs (approximately X2), even though dendritic complexity in terms of the number of branches appeared to be comparable between genotypes. Similarly total number of spines was also higher in TgBDNF compared to WT granule neurons, albeit spine density did not change between genotypes. These results suggest that excess BDNF may be associated with increased synaptic input from entorhinal cortex onto granule neuron dendritic arbors as evidenced by increased dendritic length and number of spines compared to WT levels. This increase in cortical projections onto granule neurons in TgBDNF mice may be reflected in enlarged MF terminal fields as the information processing progresses in the trisynaptic circuitry. Whether the excess BDNF-related synaptic alterations include changes in the CA3 dendritic receptive fields is currently being assessed. These studies map out the early circuit disruptions in the hippocampus of the TgBDNF mice.

Disclosures: C. Isgor: None. **F. Hossain:** None. **C. Aydin:** None. **O. Oztan:** None. **K. Guthrie:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.14/X5

Topic: C.08. Epilepsy

Support: NINDS R01-NS-065020

Title: Threshold for granule cell mediated epileptogenisis

Authors: *I. J. ROLLE[1], B. KESTLER[1], R. PUN[2], S. DANZER[2];
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Abstract: Animal and human studies of temporal lobe epilepsy have long implicated dentate granule cells (DGCs) as mediating many epileptogenic changes. Previously, we demonstrated that selective deletion of phosphatase and tensin homologue (PTEN) from 10-25% of DGCs was sufficient to produce a profound epilepsy syndrome (Pun et al., Neuron, 2012). PTEN deletion from granule cells leads to hyperactivation of the mammalian target of rapamycin (mTOR) pathway, and our PTEN knockout model reproduces abnormalities observed in temporal lobe epilepsy. These findings support the hypothesis that accumulation of abnormal granule cells may mediate temporal lobe epileptogenesis. Although abnormal granule cells are characteristic of temporal lobe epilepsy, the percentage of abnormal cells in epilepsy is often much less than 25%. To better assess the clinical significance of abnormal cells, therefore, we designed a protocol to delete PTEN from just ≈5% of DGCs. Animals were monitored 24/7 by video-EEG to quantify seizure onset and frequency. Strikingly, animals in which PTEN was deleted from ≈5% of DGCs exhibited spontaneous recurrent seizures, demonstrating that a "load" of abnormal granule cells similar to that observed in status epilepticus models of epilepsy is sufficient to generate spontaneous seizures. Interesting, the disease was less severe in mice with ≈5% PTEN KO DGC relative to animals in our previous study with 10-25% PTEN KO DGC, with later onset of seizures and reduced mortality. These findings suggest that epilepsy phenotype and disease progression may be dependent upon the number of abnormal granule cells.

Disclosures: I.J. Rolle: None. **B. Kestler:** None. **R. Pun:** None. **S. Danzer:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.15/X6

Topic: C.08. Epilepsy

Support: Intramural Research Programs of NIMH

Grant-in-Aid for Scientific Research of Ministry of Education, Culture, Sports, Science and Technology, Japan (Grant #: 22591274)

Title: Selective loss of hilar mossy cells increases dentate excitability

Authors: *S. JINDE[1], V. ZSIROS[2], K. NAKAO[2], K. NAKAZAWA[2];
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Abstract: Glutamatergic hilar mossy cells of the dentate gyrus can either excite or inhibit distant granule cells, depending on whether their direct excitatory projections to granule cells or their projections to local inhibitory interneurons dominate. However, it remains controversial whether the net effect of mossy cell loss is granule cell excitation or inhibition. To explore their influence on dentate excitability and hippocampal function, we first generated a new transgenic mouse line, floxed-diphtheria toxin receptor (DTR)-line B, in which DTR is expressed under the control of the calcium/calmodulin-dependent protein kinase II (CaMKII)-alpha promoter following Cre-mediated recombination. We then crossed the line with a mossy cell/CA3 Cre line (Cre #4688) to generate double transgenic mutants in which DTR is expressed exclusively in dentate mossy cells. We have already presented in previous SfN meetings that the mutant mice, which received i.p. injections of diphtheria toxin (DT) for two consecutive days, showed selective mossy cell degeneration followed by a significant gamma-aminobutyric acid (GABA)-ergic sprouting, but not mossy fiber sprouting, in the dentate inner molecular layer (IML) by 4 weeks after treatment (chronic phase), and the impairment of pattern separation especially in first one week after treatment (acute phase). In this study, we present that the injection of toxin into this mutant induced an extensive degeneration of mossy cells throughout the longitudinal axis, accompanied by the transient increase of theta wave power of dentate local field potentials during exploration and kainic acid-induced immediate early gene expression in dentate granule cells during acute phase. By contrast, we detected no epileptiform activity or spontaneous behavioral seizures after mossy cell degeneration. These results indicate that the net effect of mossy cell excitation is to inhibit granule cell activity.

Disclosures: S. Jinde: None. **V. Zsiros:** None. **K. Nakao:** None. **K. Nakazawa:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.16/X7

Topic: C.08. Epilepsy

Support: Epilepsy Foundation grant

Baylor University Research Council grant

Title: Early-life seizures result in long-term elevation in anxiety in mice and spatial learning deficits

Authors: *N. AHMED[1], G. SMITH[2], E. ARBUCKLE[2], J. N. LUGO[3];
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Abstract: One of the most devastating aspects of developmental epilepsy is the long-term impact on behavior. Children with epilepsy show a high co-morbidity with psychiatric disorders. To examine whether early-life seizures result in alterations in anxiety and learning and memory we administered the chemoconvulsant kainic acid to induce seizures in C57BL/6 male mice. The mice received an injection of 2 mg/kg (intraperitoneal) of kainic acid. The treatment induced status epilepticus in postnatal day 10 pups for approximately 1.5 hrs. There was a parallel saline injected group and a naïve control group in addition to the kainic acid treated mice. The subjects were then tested in a battery of behavioral tests in adulthood: open field activity, elevated-plus maze, light-dark test, conditioned fear, novel object recognition, and Morris water maze. The mice with early-life seizures showed a consistent increase in anxiety in all three behavioral tests that measure changes in anxiety. The mice with early-life status epilepticus showed a decrease in time in the center of an open field test ($p < 0.05$) without alterations in total activity. They showed decrease time in the open arms of the plus-maze test ($p < 0.05$) and increased time in the closed arms ($p < 0.05$). The seizure mice did not show a difference in center time or in total distance traveled in the elevated plus maze compared to controls. In the light-dark test the mice with early-life seizures showed less transitions between the light to dark areas compared to the controls ($p < 0.05$). We then tested the groups in a standard fear conditioning test found no differences in freezing compared to controls. There were no differences in freezing to the conditioned stimulus (tone) or to the context. The mice with seizures also had no deficits in short-term memory in the novel-object recognition test. However, the mice with early-life seizures had a deficit in spatial learning. They had a longer latency to reach the hidden platform across the 8 trials of testing ($p < 0.01$). They also spent less time in the quadrant that originally housed the hidden platform during the probe trail ($p < 0.05$). The seizure mice appear to have alterations in anxiety and in spatial learning. We did not observe any spontaneous seizures during the testing period. These results demonstrate that mice with one insult of status epilepticus on postnatal day 10 have a long-lasting increase in anxiety and spatial learning.

Disclosures: N. Ahmed: None. **G. Smith:** None. **E. Arbuckle:** None. **J.N. Lugo:** None.

Poster

536. **Epilepsy: Hippocampus and Learning Disorders**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 536.17/X8

Topic: C.08. Epilepsy

Support: NIH (NINDS RO1 NS32403 & NS38572) DAC

NIH (NIGMS IRACDA GM081259) SFF

Title: Lateral and medial perforant path inputs activate distinct populations of hippocampal dentate granule cells

Authors: *S. F. FRAUSTO[1,2], C. YUE[1], C. G. DENGLER[3,1], H. TAKANO[1], D. A. COULTER[1,3,4];
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Abstract: The dentate gyrus (DG) is a microcircuit that is essential to the cognitive processes of the hippocampus. It has been implicated in pattern separation and pattern completion, both of which are crucial to the formation of episodic memories. Dentate granule cells (DGCs) exhibit sparse activation of distinct subsets of populations during completion of cognitive tasks *in vivo*. The principal inputs to the DG stem from the entorhinal cortex via the perforant path (PP). The PP is composed of the medial (MPP) and lateral perforant path (LPP). The medial enthorinal cortex relays spatial information through the MPP, while the lateral enthorinal cortex encodes non-spatial information. However, it is unclear how processing of information through these distinct pathways contributes to sparse activation of DGCs. In this study, we selectively activated the two components of the PP to assess their contributions to proportional activation of DGCs. We utilized in vitro multicellular calcium imaging (MCI) combined with voltage sensitive dye imaging (VSDI) techniques to examine in detail the mechanisms mediating firing specificity of DGCs in response to MPP or LPP afferent stimulation at theta frequency (5 Hz) in hippocampal entorhinal-cortex slices from adult mice (8-12 wks). We have previously reported that there is a significant overlap in DGCs activating mutually to two distinct PP stimuli, suggesting deterministic firing. We found that sparse activation of DGCs was maintained upon stimulation of PP inputs (15.2% for MPP and 25.1% for LPP, n=228 cells). Interestingly, we observed only 30% overlap in the population of DGCs that were activated mutually to MPP and LPP stimuli, with 70% of cells responding to only one input. We tested the ability of DCG-IV, a group II mGluR agonist, to selectively block the activation of PP inputs. Surprisingly, application of DGC-IV had no preferential selectivity on MPP or LPP, effectively decreasing DGC activation to both inputs (91.2% and 85.6%, respectively). DG VSDI recorded perforant path evoked EPSPs were also reduced after the application of DCG-IV. Furthermore, hilar activation was completely abolished, suggesting a loss of activation of downstream efferent structures.
In summary, we found that activation of DGCs by MPP and LPP inputs remained sparse and that the MPP and LPP activated unique DGC ensembles. These results provide insight into the

network activation properties of the normal DG and how they may contribute to cognitive functions.
Research supported by NINDS (RO1 NS32403 & NS38572) to DAC and Penn-PORT (NIH IRACDA, GM081259-05) Fellowship to SFF.

Disclosures: S.F. Frausto: None. **C. Yue:** None. **C.G. Dengler:** None. **H. Takano:** None. **D.A. Coulter:** None.**Poster**

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.01/X9

Topic: C.21.Perinatal Brain Injury

Support: a Grant-in-Aid for Scientific Research (JSPS KAKENHI 24591617) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: A novel reproducible model of neonatal stroke in mice: Comparison with a hypoxia-ischemia model

Authors: *M. TSUJI[1], M. OHSHIMA[1], A. TAGUCHI[2], Y. KASAHARA[2], T. IKEDA[3], T. MATSUYAMA[4];
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Abstract: Background & Objectives: Neonatal stroke occurs in 1/4000 live births and leaves life-long neurological impairments, such as cerebral palsy. Currently, the rodent models of neonatal stroke that are available exhibit significant inter-animal variability, which makes it difficult to accurately assess the mechanisms of brain injury and the efficacy of candidate treatments. We aimed to introduce a novel, highly reproducible model of stroke, middle cerebral artery occlusion (MCAO), in immature mice, and to evaluate the reproducibility of this model compared with a conventional hypoxia-ischemia (HI) model.
Methods: Postnatal day 12 (P12) male and female CB-17 mouse pups were prepared for surgery. Permanent MCAO was produced by directly electrocauterizing the left MCA. HI was induced by a combination of permanent occlusion of the left common carotid artery and systemic hypoxia. The cortical surface cerebral blood flow (CBF) was measured by a laser speckle flowmetry imaging system. Animal behaviors were evaluated using the rotarod test and the open-field test. Morphological evaluation of the brain injury was performed at 48 hours after the insult using

TTC staining, and at 8 weeks after the insult using the H&E staining.
Results: The MCAO model exhibited excellent long-term survival; 85% up to 8 weeks after the insult. Infarct was evident in every animal with MCAO (n = 27) and was confined to the cortex, with the exception of some mild thalamic injury. While the % stroke volume 48h after the insult was consistent in the MCAO group, range: 17.8-30.4% (minimum-maximum), it was substantially less consistent in the HI group, range: 3.0-70.1%. This contrasting variability between the two models was also evident in the CBF, 24h after the insult, and in the ipsilateral hemispheric volume, as assessed at 8 weeks after the insult. Mice with MCAO exhibited significant neurofunctional deficits in the rotarod and open-field tests.
Conclusions: Preclinical studies for neonatal stroke could become more reliable using this model, with even a potential reduction in the number of pups required for statistical significance. The contrasting variability between the two models may provide insights into the factors that contribute to inter-animal variability in brain injury.

Disclosures: M. Tsuji: None. **M. Ohshima:** None. **A. Taguchi:** None. **Y. Kasahara:** None. **T. Ikeda:** None. **T. Matsuyama:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.02/X10

Topic: C.09. Ischemia

Support: NIH-NINDS NS-066001

NIH-NINDS NS-055832

Title: A critical role for cortical activation in protection from ischemic stroke damage

Authors: *M. F. DAVIS, C. H. CHEN-BEE, R. D. FROSTIG;
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Abstract: Our lab has previously demonstrated that complete protection from impending ischemic stroke damage can be achieved if single whisker stimulation is delivered within the first 2 hours following ischemic onset in a rodent model of permanent middle cerebral artery occlusion (pMCAO). Reperfusion was achieved via stimulation induced collateral vessel flow into MCA branches. Given that animal and clinical research suggest that reperfusion alone does not necessarily result in complete protection from ischemia, we hypothesized that evoked cortical activity (resulting from sensory stimulation) might play role, in addition to initiating

blood flow return, in achieving more effective protection from impending stroke damage. In the current study we sought to separate the variables of reperfusion and cortical activity by using a removable artery clamp to temporarily occlude middle cerebral artery (MCA) for one hour (1h tMCAO). In this way we ensured that equivalent reperfusion occurred in both experimental and un-stimulated control animals at the same post-occlusion time point and could assess the effect of adding sensory stimulation evoked cortical activity. We assessed blood flow using laser speckle imaging throughout the experiment and stained for subsequent infarct using TTC in stimulated (1h tMCAO with stim; n=17) and un-stimulated (1h tMCAO NO stim; n=12) animals. We demonstrate that blood flow drop, reperfusion level, and blood flow the following day is identical between groups. Despite identical reperfusion between groups, the addition of single whisker stimulation evoked cortical activity significantly reduced damage in stimulated compared to un-stimulated animals (1h tMCAO with stim: mean = 12.5+1.9mm3 vs 1h tMCAO NO stim: mean = 24.0+3.9mm3; paired t-test $t(29)= 3.71$; $p< 0.0009$). Further analysis suggests that evoking cortical activity within the ischemic cortical region might temper the damaging ability of reperfusion. In 1h tMCAO NO stim control animals, increased reperfusion values following clamp release were correlated with increased infarct size (Pearson's correlation [two tailed] [$r = 0.633$, $n =12$, $p<0.05$]), whereas in 1h tMCAO with stim animals, increased reperfusion values were not correlated with increased infarct size and in fact there was a trend towards increased reperfusion values being correlated with decreased infarct sizes. If translational, stimulation based treatment could provide a means to augment current blood flow re-establishment strategies and perhaps lead to outcomes more similar to the complete protection observed in our rodent pMCAO model.

Disclosures: M.F. Davis: None. **C.H. Chen-Bee:** None. **R.D. Frostig:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.03/X11

Topic: C.09. Ischemia

Support: CIHR - Operating Grant

Title: Investigating the association between reduced CVR, cortical thinning and cognitive deficits in the pediatric population with sickle cell disease

Authors: *J. KIM[1,3], J. LEUNG[3], J. LERCH[4], G. DEVEBER[4], B. NIEMAN*[4], A. KASSNER[3,2];

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Abstract: Background: Sickle cell disease (SCD) is a genetic disorder that is known to affect normal physiological and neurological development. Children with SCD suffer from cognitive deficits even with brains that are assessed as normal with conventional Magnetic Resonance Imaging (MRI). Cerebrovascular reactivity (CVR), defined as the percent change in cerebral blood flow in response to a CO_2 stimulus, is determined with the use of blood-oxygen level-dependent (BOLD) MRI sequence. CVR can be utilized to gauge cerebrovascular health and therefore CVR can aid with the clinical assessment of SCD patients. In non SCD patients, brain regions with reduced CVR have previously been correlated to cortical thinning. However, it is unknown whether compromised CVR in SCD has a direct physiological impact on cortical integrity and could serve as a biomarker of cognitive decline.
Objective: The purpose of this study was to assess if reduced CVR is associated with cortical thinning in children with SCD.
Patients and Methods: 40 SCD patients (12-18 years) and 15 controls were imaged on a 3T MRI. Anatomical and CVR data were acquired. For CVR, a BOLD sequence was used during a computer-controlled CO_2 stimulus. CVR maps were computed by correlating the voxel-wise BOLD signal changes to the end-tidal CO_2 waveform, then coregistered to the anatomical space. Cortical thickness was computed from the anatomical data using the CIVET pipeline and a correlation analysis between thickness and CVR was performed within each functional region of the brain using the MATLAB based toolkit SurfStat.
Result: Group analysis between patients and controls revealed multiple regions with significant reduction in both cortical thickness and CVR in SCD patients. Some highlighted correlations included strong correlations in the right post central gyrus ($r = 0.5996$), moderate to significant correlation in the right orbital frontal cortex ($r = 0.5328$) and moderate correlations in the bilateral cuneus ($r = 0.4311$ left, $r = 0.4038$ right).
Discussion: In this study, we have demonstrated a linear relation between the degree of cortical thinning and reduced CVR in specific regions of the brain within the pediatric SCD population. This finding can potentially indicate that certain cortical areas are increasingly susceptible to disruptions in normal blood flow regulation leading to poor maintenance of the structural integrity in these areas. Future objectives will include measures of cognitive ability, which will be correlated with CVR and cortical thickness data in order to investigate the relationship between cognitive deficits, CVR and cortical thinning.

Disclosures: J. Kim: None. **J. Leung:** None. **J. Lerch:** None. **G. deVeber:** None. **B. Nieman*:** None. **A. Kassner:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.04/X12

Topic: C.09. Ischemia

Support: NIH Grant GM49831 to RGG

NIH Grant NS053898 to RGG

NIH Grant GM089626 to CMS

Title: Role for microRNA-200c in response to ischemia

Authors: C. M. STARY, L. XU, Y.-B. OUYANG, J.-M. MOON, *R. G. GIFFARD;
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Abstract: Micro-RNAs (miRs) are small non-coding RNAs that inhibit the translation of target mRNAs. Homology sequence searches suggest miR-200c may target genes that regulate cell survival. We hypothesized that miR-200c is a determinant of survival following cerebral ischemia by modulating the cellular response to ischemia. We investigated its effect on in vitro ischemic injury, and whether expression of miR-200c changed in response to focal ischemia. Candidate pro-survival genes (GRP78 and BCL2) were identified using Targetscan.org. For in vitro experiments, mouse neuronal and astrocyte cultures were transfected with miR-200c mimic or inhibitor and subjected to oxygen-glucose deprivation, followed by 24 hrs reperfusion. Levels of miR-200c were assessed by RT-qPCR and GRP78 and BCL2 protein levels by western blot. Cell death was assessed by lactate dehydrogenase release. Baseline levels of miR-200c in naïve astrocyte and neuronal cultures were comparable. Treatment with miR-200c mimic and inhibitor effectively increased and decreased miR-200c levels respectively in cell culture. Astrocytes treated with miR-200c inhibitor exhibited greater Grp78 and Bcl2 protein levels. When stressed by oxygen-glucose deprivation, pretreatment with inhibitor improved astrocyte cell survival. Treatment of neuronal cultures with miR-200c mimic exacerbated cell death. For in vivo experiments, adult male C57B6 mice were subjected to 1 hr MCAO and 1 hr reperfusion, followed by assessment of miR-200c levels. In brains of mice subjected to MCAO, miR-200c increased several fold. The results of this study demonstrate that the use of mimic and inhibitor alters levels of miR-200c, and levels of Grp78 and Bcl2 protein. This results in increased injury in vitro with mimic, and decreased injury with inhibitor. Further, miR-200c increases in response to focal ischemia. These findings suggest that the increase in miR-200c that accompanies cerebral ischemia may exacerbate brain injury by reducing levels of cell-survival proteins Grp78 and Bcl2. This suggests targeting miR-200 could be a future therapeutic candidate for stroke.

Disclosures: C.M. Stary: None. **R.G. Giffard:** None. **L. Xu:** None. **Y. Ouyang:** None. **J. Moon:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.05/X13

Topic: C.21.Perinatal Brain Injury

Support: NINDS R01-NS060765

Baby Alex Foundation

Title: Erythropoietin attenuates KCC2 chloride cotransporter loss and promotes recovery following *In utero* hypoxia-ischemia

Authors: *L. L. JANTZIE[1], D. J. FIRL[2], S. ROBINSON[2];
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Abstract: Children with perinatal brain injury are prone to neuropsychiatric disabilities including cognitive disorders, learning disabilities, autism and epilepsy. During development, the potassium chloride co-transporter KCC2 is upregulated and directs the formation of essential inhibitory circuits by driving $GABA_A$ receptor activation to hyperpolarization. Loss of KCC2 is observed in preterm infants with brain injury and resected epilepsy tissue. Here, we investigated how late gestation hypoxia-ischemia alters KCC2 upregulation and impairs the formation of essential inhibitory circuits. We hypothesized that KCC2 would be chronically decreased following prenatal brain injury via calpain-dependent mechanisms and that postnatal treatment with erythropoietin (EPO) would protect the developing brain and reverse KCC2 loss. Accordingly, prenatal *in utero* transient systemic hypoxia-ischemia (TSHI) was performed on embryonic day 18 (E18) rats to mimic brain injury in infants born extremely preterm. Following TSHI, EPO or vehicle was administered from postnatal day P1-5 (2000 IU/kg/dose/ip). KCC2 mRNA and protein were assessed in microdissected CA3 from P2-P28 ($n \geq 8$/group for all). After TSHI, functional oligomeric KCC2 was reduced by 13% compared to sham (0.87±0.034 vs. 1.01±0.0.03, $p<0.001$). Loss of oligomeric KCC2 persisted through P28, with TSHI KCC2 expression 18% below shams (0.82±0.03 vs. 1.01±0.05, $p=0.002$). Along with this loss in KCC2, TSHI significantly increased 90 and 30 kD KCC2 fragmentation at P11 and μ-calpain activity by 59%, as measured by the ratio of cleaved to full length αII-spectrin (1.59±0.11 vs.1.00 ±0.04, $p=0.005$). Neonatal EPO treatment normalized KCC2 oligomer levels at P11 (1.11±0.06, two-way ANOVA $p<0.001$) and P28 (0.995±0.03) suggesting early neonatal EPO treatment restores

KCC2 oligomer levels in young adult brains. EPO also decreased the αII-spectrin ratio following TSHI by 44% (p=0.01). In sum, prenatal brain injury limits the developmental increase of KCC2 protein expression. Moreover, postnatal administration of EPO reverses KCC2 loss through P28, attenuates KCC2 oligomeric fragmentation and normalizes μ-calpain activity following TSHI. These data indicate it is possible to reverse abnormalities in inhibitory circuit development in the postnatal period. KCC2 loss may be essential to the molecular pathophysiology of late gestation brain insults, and EPO treatment is a viable and clinically relevant therapeutic strategy in this patient population.

Disclosures: L.L. Jantzie: None. **D.J. Firl:** None. **S. Robinson:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.06/X14

Topic: C.09. Ischemia

Support: 09KW-11, Department of Health, State of Florida

Title: Neuroprotective effects of Taurine and S-methyl-N, N diethylthiocarbamate sulfoxide on rat middle cerebral artery occlusion stroke model

Authors: *P. MOHAMMAD GHARIBANI, J. MODI, J. MENZI, H. PRENTICE, J.-Y. WU; Florida Atlantic Univ., Boca Raton, FL

Abstract: Taurine has been shown to provide protection against neurological diseases, such as Huntington's and stroke. On the other hand, we have shown in our previous study that either taurine (40 mg/kg) or S-methyl-N, Ndiethylthiocarbamate sulfoxide (DETC-MeSO) (5.6 mg/kg) could attenuate glutamate excitotoxicity and protect against Endoplasmic Reticulum (ER) stress in the model of Middle Cerebral Artery Occlusion (MCAO). In this study, we employed combination of taurine and DETC-MeSO with a lower dose (0.56 mg/kg) for in vivo model of rat MCAO. Using taurine greatly decreased the infarct area and levels of the ER stress proteins p-IRE-1 and cleaved ATF6. On the other hand, taurine induced an up-regulation of the Bcl-2/Bax ratio and downregulation of Caspase-3 protein activity. DETC-MeSO in a lower dose almost did not show any changes on ER stress. In contrast, when it was administered with the combination of taurine, not only reduced infarct size significantly less than administering taurine individually but also it decreased αII-spectrin cleavage and the level of ATF4 protein expression in PERK pathway. Our results show not only taurine elicits neuroprotection through the

activation of the ATF6 and the IRE1 pathways, but also in combination with DETC-MeSO with lower dose can inhibit all 3 pathways of ER stress besides apoptosis.

Disclosures: P. Mohammad Gharibani: None. **J. Modi:** None. **J. Menzi:** None. **H. Prentice:** None. **J. Wu:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.07/X15

Topic: C.09. Ischemia

Support: 1R01HD070996

1R21NS059529

Title: Regional and gender specific hypothermic neuroprotection in a neonatal mouse model of hypoxic-ischemic injury

Authors: J. C. BURNSED[1], J. ZHANG[2], R. CHAVEZ-VALDEZ[1], K. KESAVAN[1], L. J. MARTIN[3], *F. J. NORTHINGTON[1];
[1]Pediatrics and Neonatalogy, [2]Radiology and Neuroimaging, [3]Pathology and Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Background: Hypothermia (HT) is standard treatment for infants with moderate to severe hypoxic ischemic injury (HI); however, HT is not completely protective. Additionally, there is well described regional vulnerability to injury in the immature brain and gender biased differences in susceptibility to injury and response to therapy (Hill 2011). Studies examining MRI of infants receiving HT or normothermia suggest that HT may have differential regional protection (Inder 2004, Rutherford 2005, Bonafacio 2011). There is not a well established neonatal murine model of HT following neonatal HI. Objectives:To develop a reproducible model of HT in neonatal mouse model of HI, determine whether HT provides regional and gender specific patterns of neuroprotection. Design/Methods: HI was created in postnatal day 10 (p10) C57BL6 mice using the Vannucci model (unilateral carotid ligation+45 minutes FiO2=0.08). Mice were randomized to HT (31ºC) or normothermia (36ºC) for 4 hours immediately following HI. T2-weighted MRI was obtained on one cohort of mice at p11 and p18. Regions of interest (cortex, thalamus, striatum, and hippocampus), total lesion and brain volumes were measured on T2-weighted images. Fresh tissue from another cohort of mice was obtained at p11 for protein activity assay of complex I and caspase 3 and Western blot for

nitrotyrosine. Data were analyzed using a Mann-Whitney U test and one-way ANOVA. Results: Total injury volume in males was significantly decreased at p18 in the hypothermia group on T2-weighted images (Mann Whitney U Test: p=0.05). In males, HI appears to impair brain growth from p11 to p18, this effect appears to be reversed by 4 hours of 31°C hypothermia (One-way ANOVA, p=0.002;post-hoc (Tamhane), p=0.04 (control vs. normothermia), p=0.006 (normothermia vs. HT)). Regional injury volumes in males show significant neuroprotection at p18 in cortex and hippocampus (Mann Whitney U Test: p=0.05 (n=3) p=0.006 (normothermia vs. HT)). Caspase-3 activity (One-way ANOVA, p=0.012; p=0.01 vs. control; p=0.05 vs. normothermia (Tukey post-hoc test) and nitrotyrosine labeling is increased and complex I activity is decreased **(**One-way ANOVA, p=0.04, p=0.05 vs. hypothermia (Tukey post-hoc test), in forebrain of normothermic animals compared to hypothermia and control groups. Conclusions: Hypothermia following HI provides measureable regional neuroprotection and prevents evolution of injury from 1-7 days in p10 male mice. Hypothermia in males may provide specific mitochondrial protection. Whether the same is true in females and studies to determine whether these early findings impact on long term structural and functional outcomes is underway.

Disclosures: J.C. Burnsed: None. **J. Zhang:** None. **R. Chavez-Valdez:** None. **K. Kesavan:** None. **F.J. Northington:** None. **L.J. Martin:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.08/X16

Topic: C.09. Ischemia

Support: NS079153 (PI: F. Sharp)

NS070535 (PI: F. Sharp)

Grant funding from NINDS to do the CLEAR-ER Trial (A. P.)

Part of J.K.'s salary was paid on the SPOTRIAS grant .

Title: The initial inflammatory response may predict long-term functional outcome in stroke

Authors: *B. STAMOVA[1], G. JICKLING[1], B. ANDER[1], X. ZHAN[1], D. LIU[1], J. KHOURY[2], A. PANCIOLI[3], E. JAUCH[4], J. P. BRODERICK[3], F. R. SHARP[1];
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Biostatistics and Epidemiology, Cincinnati Children's Hosp. Med. Center,, Cincinnati, OH; [3]Neurol., Univ. of Cincinnati, Cincinnati, OH; [4]Div. of Emergency Med., Med. Univ. of South Carolina, Charleston, SC

Abstract: BACKGROUND AND PURPOSE:
The immune and clotting systems play a major role in causing stroke and affecting outcomes. Leukocytes detect blood clots in the vascular system, damaged brain endothelium, atherosclerotic plaque and platelets adhering to vessel walls, and leukocytes modulate clotting. This study aimed to (1) develop a pilot profile of genes, whose early post-stroke expression associates with late (5-day and 90-day) functional outcome; and (2) identify the early inflammatory pathways involved in long-term functional outcome in stroke.
METHODS:
Subjects with ischemic stroke (IS) from the CLEAR trial (NCT00250991) were analyzed (65.8+/-13.5 years). A Derivation Set of patients with IS (n=35 and n=26 for 5-day and 90-day outcome, respectively), and a Validation Set (n=15 and n=11 for 5-day and 90-day outcome, respectively) were evaluated for late stroke outcome prediction. Whole blood was drawn ≤3 hours after stroke onset (no treatment) and RNA processed on whole genome microarrays. Pearson correlations between gene expression and NIH Stroke Scale (NIHSS) at 5 days and 90 days, after adjusting for batch, age and biological sex, were determined. Genes from the top 20 positive and the top 20 negative correlations (FDR<0.001, |r>0.9|) in the Derivation Set were used to generate a Support Vector Machine Regression model, which was tested on the Validation Set.
RESULTS:
The top 40 genes identified to correlate the strongest with 5-day and 90-day NIHSS predicted outcome in the Validation set with R^2=0.98 and 0.96, respectively. There was no overlap of the 5-day and 90-day predictor genes. The top pathways over-represented in the top 200 genes (FDR p<0.001, |r|>0.7), whose expression correlates with 5-day outcome was sphingosine-1-phosphate signaling (FDR p=0.002), implicated in stroke and progression of disease. The top 90-day pathway was Integrin Signaling (FDR p=0.2), implicated in stroke risk and in functional outcomes following stroke. Predicted upstream activation of TGFB1, TGFB3, AGT (Angiotensinogen), and VEGF growth factors, and inhibition of ABCB2 transporter and hypoxia-inducible factor1 alpha (HIF1a) expression at 3h, correlated with 90-day outcome. Several of these have been shown to affect stroke outcome in animal models and humans.
CONCLUSIONS:
This pilot study adds to the evidence that the immune response after stroke may influence later functional outcome. It will expand our understanding of the genomic underpinnings associated with stroke outcome. These findings need to be validated in a larger sample and in an independent cohort. Additional studies associating gene expression with infarct volume and outcome sub-scales is underway.

Disclosures: B. Stamova: None. **G. Jickling:** None. **B. Ander:** None. **X. Zhan:** None. **D. Liu:** None. **J. Khoury:** None. **A. Pancioli:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); study drug for CLEAR-ER from Merck and Genentech.. **E. Jauch:** None. **J.P. Broderick:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Genentech Inc. (Supplier of alteplase for NINDS-funded CLEARER, IMS III trials), Novo Nordisk (Supplier of drug for NINDS-funded STOP-IT trial, Schering Plough supplies drug for NINDS-funded CLEARER Trial.. Other; $65,000 educational grant to the American Academy of Neurology for 2012 annual meeting program 2AC.007 "What's in a Stroke Center: Members, Services, Organization and Roles" which I directed.. **F.R. Sharp:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.10/X18

Topic: C.09. Ischemia

Title: Possible relationship between decreased expression of lysosomal-associated membrane protein type 2A and delayed neuronal death after brain ischemia

Authors: *E. DOHI[1,2], S. TANAKA[1,2], T. SEKI[2], T. MIYAGI[2], I. HIDE[2], T. TAKAHASHI[1], M. MATSUMOTO[1], N. SAKAI[2];
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Abstract: Autophagy is a conserved mechanism responsible for the continuous clearance of unnecessary organelles or misfolded proteins in lysosomes. Three types of autophagy have been reported in the difference of substrate delivery to lysosome: macroautophagy (MA), microautophagy, and chaperone-mediated autophagy (CMA). Among these types, CMA is a unique autophagy system that selectively degrades substrates detected by heat shock cognate protein 70 (HSC70). Substrates are then transferred to the lysosomal membrane through an interaction with lysosomal-associated membrane protein type 2A (LAMP-2A), thereby translocated into the lysosome. Recently, we have reported that CMA is activated during hypoxia and contributes to the survival of cells under these conditions (Dohi E et al., Neurochem Int. 2012). In the current study, we asked whether CMA would be activated in neurons after brain ischemia in vivo. Male Wistar rats, 7 weeks of age, were subjected to permanent middle cerebral artery occlusion (pMCAO) and LAMP-2A expression after cerebral ischemia was evaluated by both westernblotting and immunohistochemistry. LAMP-2A expression in the lysosomal fraction was decreased at day 2, compared with that in sham control, and then it was increased until day 7

in the ipsilateral hemisphere. In the contralateral hemisphere, LAMP-2A expression in the lysosomal fraction was slightly increased at day 4 and then returned to the basal level at day 7. Besides, the LAMP-2A staining in the ischemic brain revealed that LAMP-2A expression in neurons was decreased in the ischemic hemisphere from 1 day to 2 days after pMCAO. However, at 7 days after pMCAO, cortical neurons in the peri-infarct area indicated stronger LAMP-2A expression, compared with the contra-lateral side. We further evaluated LAMP-2A expression using the rat four-vessel occlusion (4VO) model (Pulsinelli et al., 1982). LAMP-2A expression was decreased mainly in the vulnerable CA1 neurons of hippocampus 2 days after ischemia. These results suggested that hampered CMA activity in the ischemic vulnerable neurons might be associated with delayed neuronal death after brain ischemia.

Disclosures: E. Dohi: None. **S. Tanaka:** None. **T. Seki:** None. **T. Miyagi:** None. **I. Hide:** None. **T. Takahashi:** None. **M. Matsumoto:** None. **N. Sakai:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.11/Y1

Topic: C.09. Ischemia

Support: NSERC

Innovation PEI

Atlantic Innovation Fund

Title: Effects of focal ischemic lesions of the prefrontal cortex on cognition in the rat

Authors: *R. A. DÉZIEL, R. A. TASKER;
Univ. of Prince Edward Island, Charlottetown, PE, Canada

Abstract: Stroke is the most common cause of long-term disability in adults, and it has been estimated that approximately 30% of stroke survivors experience cognitive impairments persisting for at least three months post-stroke. As well, approximately half of all cases of dementia may be wholly or in part due to vascular impairment. Many cognitive deficits in stroke survivors occur because of damage to the prefrontal cortex (PFC), which has been heavily implicated as the seat of executive function in the brain. There is a need, therefore, for an improved understanding of the role of the PFC in executive function deficits, as well as the role of neuroplasticity in cognitive recovery. Currently, there are few rat models examining prefrontal

cognitive dysfunction following stroke and none that utilize focal ischemic lesions specifically in the PFC. Utilizing bilateral microinjections (1 ul) of the vasoconstricting peptide endothelin-1 (ET-1) (400 pmol) into the medial PFC in adult male SD rats (N=12/group) we produced highly localized ischemic lesions in the PFC (or sham) and the effects of these lesions on cognition were assessed using a test of temporal order memory and a colour-texture discrimination attentional set-shifting task. Following testing rats were euthanized and lesion location and volume was confirmed histologically. The combination of focal damage to a specialized cortical region and behavioural tests of executive function shows potential for developing a model of post-ischemic cognitive dysfunction that will support subsequent studies of cognitive rehabilitation and cortical neuroplasticity.

Disclosures: R.A. Déziel: None. **R.A. Tasker:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.12/Y2

Topic: C.09. Ischemia

Support: Grants from the National Basic Research Program of China 2013CB910204 to WY

Grants from the National Basic Research Program of China 2010CB912002 to JHL

Royal Society to WY and L-HJ

Alzheimer Research Trust to L-HJ

Title: TRPM2 contributes to ischemia-induced delayed neuronal death in hippocampal CA1 region and impairment in motor activity via disrupting zinc homeostasis

Authors: *M. YE[1], W. YANG[1], W.-Y. YU[1], X.-H. ZHANG[1], L.-H. JIANG[2], J.-H. LUO[1]; [1]Dept. of Neurobio., Inst. of Neuroscience, Zhejiang Univ. Sch. of Med., Zhejiang Province, China; [2]Sch. of Biomed. Sciences, Fac. of Biol. Sciences, Univ. of Leeds, Leeds LS2 9JT, United Kingdom

Abstract: Oxidative stress (OS) is a major factor in ischemia-induced neurological damage.TRPM2 is a nonselective cation melastatin-related transient receptor potential channel activated by reactive oxygen species (ROS), and is highly expressed in the brain. Here we investigated the role of TRPM2 in transient ischemia induced delayed neuronal death in hippocampal CA1 region using wild-type and TRPM2 knock-out mice that were subjected to

transient global ischemia by bilateral common carotid arteries occlusion (BCCAO) treatment for 15 min followed by reperfusion for 72 hours. There was substantial delayed pyramidal neuronal death in the wild-type mice, which was significantly reduced in the TRPM2 KO mice. Transient global ischemia impaired motor activity in the wild-type mice, but such impairment was significantly attenuated in the TRPM2 KO mice. Disruption in zinc homeostasis has been strongly involved in OS-induced neuronal death. Parallel using confocal or two-photon microscopy experiments showed that ischemia or H2O2 significantly elevated the cytosolic zinc levels in CA1 pyramidal neurons relatively to untreated neurons which isolated from the wild-type mice. Such an ischemia induced elevation in the cytosolic zinc levels was almost completely abolished in TRPM2 deficient neurons. In summary, our results provide defining evidence to support that TRPM2 plays a significant role in ischemia-induced delayed neuronal death and impairment in motor function activity and also suggest that TRPM2-mediated disruption in zinc homeostasis may act as a novel mechanism responsible for ischemia induced neurological damage.

Disclosures: M. Ye: None. **L. Jiang:** None. **W. Yang:** None. **W. Yu:** None. **X. Zhang:** None. **J. Luo:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.13/Y3

Topic: C.09. Ischemia

Support: Maurice and Phyllis Paykel Trust Project Grant

Neurological Foundation of New Zealand Small Project Grant

W&B Miller Postgraduate Scholarship

The University of Auckland Doctoral Scholarship

Title: A novel method for inducing focal ischemia in the rat using L-N5-(1-Iminoethyl)ornithine

Authors: *A. VAN SLOOTEN[1], A. CLARKSON[2], B. CONNOR[1];
[1]Dept Pharmacol. & Clin. Pharmacology. Ctr. for Brain Res., The Univ. of Auckland, Auckland, New Zealand; [2]Depts of Anat. & Psychology, Brain Hlth. Res. Ctr., Univ. of Otago, Dunedin, New Zealand

Abstract: Stroke is a devastating neurological disorder for which new therapies are urgently needed. Animal models of stroke are a vital step in developing these treatment strategies. The middle cerebral artery occlusion (MCAo) model in rats can be highly variable and has associated mortality. We therefore sought to establish an alternative, high-throughput rat model of focal ischemia to investigate inflammation and repair in subcortical infarcts. Injection of L-N5-(1-Iminoethyl)ornithine (L-NIO), an endothelial nitric oxide synthase (eNOS) inhibitor, into the brain causes vasoconstriction and resultant ischemia. The aim of this study was to characterise the infarct, neuroinflammatory response and motor function impairments following L-NIO-induced ischemia. Male Sprague Dawley rats (300-350g) underwent right jugular vein ligation followed by L-NIO injection directly into the striatum (2µmol L-NIO in 5µl saline). Sham animals received saline injections. To assess inflammatory responses, animals were euthanized 3, 7, 14 or 35 days following L-NIO or saline injections (n=3-9/group) and immunohistochemistry performed on fixed tissue. Motor function was assessed using the cylinder test at baseline, 1 and 4 weeks post-surgery (n=7-9/group). L-NIO injection produced a consistent infarct limited to the striatum with no mortality. Although the infarct volume did not change significantly over time, atrophy of the ipsilateral striatum was observed at 35 days post-insult compared to sham ($P<0.05$). Iba1 and GFAP immunoreactivity were elevated for 35 days following L-NIO injection compared to sham surgery ($P<0.05$), demonstrating that L-NIO-induced ischemia results in microglial infiltration and initiation of reactive astrogliosis respectively. GFAP immunoreactivity was maximal 7 days following ischemia, consistent with an evolving astroglial response observed in this model. Fluoro-jade C was present within the lesion 3-7 days post-insult indicating ongoing cell death. In addition, L-NIO-induced focal ischemia resulted in impaired forelimb use on the cylinder test both 1 and 4 weeks post-insult compared to controls ($P<0.05$). We have characterised a novel method of inducing focal ischemia in rats using the eNOS inhibitor L-NIO. This model results in ongoing neuroinflammation and impaired motor function following the insult. We propose that L-NIO injection provides a consistent and high-throughput approach to model focal ischemia and future studies using this model will allow us to assess anti-inflammatory approaches to improve stroke outcomes.

Disclosures: A. Van Slooten: None. **A. Clarkson:** None. **B. Connor:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.14/Y4

Topic: C.09. Ischemia

Support: NS73666

NHMRC C.J. Martin Fellowship to KAJ

Title: Mitochondria play a role in the protective and destructive effects of chronic intermittent hypoxia on the ischemic brain

Authors: *K. A. JACKMAN, P. ZHOU, G. FARACO, T. KAHLES, C. COLEMAN, V. M. PICKEL, C. IADECOLA;
Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Severe obstructive sleep apnea, resulting in chronic intermittent hypoxia (CIH), is an independent risk factor for stroke and dementia (New Engl J Med 353(19) 2005), but the mechanisms are unknown. We have shown that mice subjected to 35 days CIH have marked dysfunction in the regulation of the cerebral circulation (Hypertens 60(1) 2011). In addition, studies in the developing brain suggest that CIH alters mitochondrial function (AJP Cell Physiol 298(6) 2010). Thus, CIH could increase stroke risk by impairing cerebral perfusion and/or mitochondria. Conversely, exposure to CIH has been reported to render the brain tolerant to ischemia (Ann Neurol 69(6) 2011). Therefore, the effects of CIH on the post-ischemic brain can be either protective or destructive, but the factors responsible for such dichotomy are undefined. We hypothesized that the severity of hypoxia during CIH is critical in determining the susceptibility of the brain to ischemic injury. Male C57BL6 mice underwent CIH (90 sec hypoxia-90 sec room air; 8 h/day during sleep) with 5% or 10% O_2 for 35 days (J Neurosci 30(36) 2010). Sham mice received room air according to the same schedule. First, we examined the effect of CIH on mitochondrial resistance to depolarization using Ca^{2+} induced depolarization of transmembrane potential ($\Delta\Psi m$). Brain mitochondria isolated from mice exposed to CIH with 10% O_2 were less susceptible to Ca^{2+} induced loss of $\Delta\Psi m$ (Ca^{2+} conc. inducing $\Delta\Psi m$ loss: Sham 4.6±0.2; CIH 5.7±0.2 nmol/mg protein; $P<0.05$; n=9), indicative of increased resistance to depolarization. In contrast, mitochondria from mice exposed to more severe CIH (5% O_2) had increased susceptibility to Ca^{2+} induced $\Delta\Psi m$ loss (Sham 5.0±0.4; CIH 2.9±0.2 nmol/mg protein; $P<0.05$; n=9). In agreement with these opposing effects on mitochondria, mice exposed to CIH with 10% O_2 were protected from brain injury produced by transient middle cerebral artery occlusion (infarct volume at 72 h: Sham 34±5; CIH 18±2 mm^3; $P<0.05$; n=10/8), while mice exposed to more severe CIH (5% O_2) had significantly larger infarcts (Sham 25±5; CIH 46±7 mm^3; $P<0.05$; n=8/10) and motor impairment (hanging wire test: Sham 24±8; CIH 7±1 sec; $P<0.05$; n=8/9). The data suggest that CIH with 10% O_2 protects the brain from cerebral ischemia, an effect associated with increased mitochondrial resistance to Ca^{2+} induced depolarization, while CIH with 5% O_2 renders mitochondria more susceptible to depolarization and the brain more vulnerable to ischemia. We conclude that the effects of CIH on ischemic brain injury are dichotomous. The greater vulnerability to ischemia induced by more severe CIH may underlie the increased stroke risk in patients with severe sleep apnea.

Disclosures: K.A. Jackman: None. **P. Zhou:** None. **G. Faraco:** None. **T. Kahles:** None. **C. Coleman:** None. **V.M. Pickel:** None. **C. Iadecola:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.15/Y5

Topic: C.09. Ischemia

Support: SFB 654

Title: Selective neuronal vulnerability of hippocampal CA1 neurones in acute neurological disorders

Authors: *T. BARTSCH[1], J. DÖHRING[1], H. BRAUER[1], J. LAGIES[1], A. ROHR[2], G. DEUSCHL[1], O. JANSEN[2];
[1]Dept. of Neurol., Univ. of Kiel, Univ. Hosp., Kiel, Germany; [2]Univ. Hosp. Schleswig-Holstein, Inst. of Neuroradiology, Kiel, Germany

Abstract: The CA1 region of hippocampus is critically involved in memory processing and learning but is also selectively vulnerable to a variety of metabolic and excitotoxic insults which can be most prominently seen in acute neurological conditions such as hypoxia-ischaemia, hypoglycaemia, encephalitis and epilepsy. The basis of this regional susceptibility is, however, poorly understood may include high demand for energy metabolism and consequent formation of reactive oxygen species in vulnerable neurons. The clinical correlates of hippocampal affection, their temporal course and sequelae have only recently been studied in vivo using magnetic resonance imaging (MRI). Here, the cellular and imaging correlates and pattern of hippocampal damage was analysed by studying the evolution of hippocampal CA1 diffusion and ADC changes in acute neurological disorders. In patients with hippocampal ischaemia (n=50), limbic encephalitis (n=30), after status epilepticus (n=17) and transient global amnesia (n=53), CA1 neurones exhibited a particular susceptibility to metabolic stress, irrespective of the nature of the insult. The evolution of diffusion changes over the course of the disease show that CA1 diffusion lesions can be most prominently detected within the first three days after the insult. Hypoxic-ischaemic insults lead to a significant lower rADC suggesting that the ischemic insult results in a stronger impairment of cellular metabolism. Studying the imaging correlates of hippocampal affection provides valuable insight into the pathophysiology and neurobiology of the hippocampus.

Disclosures: T. Bartsch: None. **J. Döhring:** None. **H. Brauer:** None. **J. Lagies:** None. **G. Deuschl:** None. **A. Rohr:** None. **O. Jansen:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.16/Y6

Topic: C.09. Ischemia

Support: Lutheran Foundation

Steel Dynamics Foundation

Title: Hsp70, hsp90 and icam-1 as early biomarkers for global cerebral ischemia

Authors: *M. JIANG[1], E. CURFMAN[1], B. C. HONG-GOKA[1,2], R. D. SWEAZEY[1], F.-L. F. CHANG[1];
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Abstract: Global cerebral ischemia caused by cardiac arrest often leads to severe brain damage, and reperfusion may further injure the brain. Currently, the only treatment to protect the brain following global ischemia is hypothermia, which has limited efficacy. Most published animal studies of global ischemia have focused on molecular events at least one day after the start of reperfusion. This leaves an information gap at earlier time points where significant damage occurs. Identification of early ischemic damage biomarkers that can be used to address this information gap is important for the development and assessment of future neuroprotective treatments. Using a global ischemia model, we have previously demonstrated increased expression of Hsp70 and ICAM-1 following 6min occlusion/3h reperfusion. Here we report results of extending ischemia and reperfusion times.
This study is in compliance with NIH animal care guidelines and was approved by the Purdue University Animal Care and Use Committee. We used a modified 4-vessel occlusion (4-VO) model in isoflurane anesthetized rats. Briefly, both vertebral arteries were exposed and electro-cauterized and the common carotid arteries were isolated and clamped for either 6 or 12min followed by 3 or 6h reperfusion. Sham animals were subjected to the same procedures except the arteries were not occluded. Cerebral blood flow and other physiological parameters were monitored. At the conclusion of the experiment, rats were sacrificed and alternate sections of brain were stored in RNAlater or immersion fixed in buffered formalin.
H&E and silver-cresyl violet double staining confirmed previous findings of damage in the

hippocampus as early as 3h of reperfusion. Similarly, mRNA and protein levels of Hsp70 and ICAM-1 were up-regulated in all stroke groups. This increase was higher in 12min/6h compared to 6min/3h or 6min/6h reperfusion groups, suggesting brain damage was mainly due to ischemia, not reperfusion, in this model. Hsp90 protein in the 6min/3h group was unchanged, but decreased in 6min/6h and 12min/6h reperfusion groups. The decrease in Hsp90 protein expression was not mirrored by mRNA levels which remained stable in all groups. The mechanism of reversed expression patterns of Hsp70 and Hsp90 need further study.
The time-dependent expression of Hsp70, Hsp90 and ICAM-1 indicates cells at risk of damage following global ischemia. Besides providing insights into the molecular events of global ischemia at these early time points, these proteins could potentially be used as biomarkers to assess drug treatment effects in our 4 VO model.

Disclosures: M. Jiang: None. **E. Curfman:** None. **B.C. Hong-Goka:** None. **R.D. Sweazey:** None. **F.F. Chang:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.17/Y7

Topic: C.09. Ischemia

Support: NIH Grant R01NS058807

KUCR startup fund

Title: Activation of HIF-1 mediates exacerbated blood-brain barrier disruption in ischemic stroke

Authors: *Z. ZHANG, H. SHI;
Univ. of Kansas, Lawrence, KS

Abstract: Hyperglycemia is associated with worsened outcomes in acute ischemic stroke. Accumulating evidence indicates that the worsened outcomes may be due to high glucose-induced cerebral vascular complications, especially disruption of the blood-brain barrier (BBB). Increased BBB permeability may contribute to the development of neurological damage in stroke. We hypothesize activation of hypoxia inducible factor-1 (HIF-1) and its target gene vascular endothelial growth factor (VEGF) is involved in hyperglycemia-aggravated BBB disruption during ischemia/reperfusion.
C57/6J mice were rendered diabetic with STZ injection. Diabetic mice and non-diabetic mice

(control) were subjected to 90 min transient middle cerebral artery occlusion (MCAO) and 24 h reperfusion. We found that there was a higher expression of HIF-1α and its target gene VEGF after MCAO/reperfusion in brain microvessels in diabetic mice than those in control animals. At the meantime, diabetic mice demonstrated exacerbated BBB damage and tight junction disruption in the ipsilateral hemisphere of the ischemic brain. Hyperglycemia also increased brain infarction and neurological deficits. Furthermore, normalizing the level of blood glucose by insulin injection abolished HIF-1α upregulation in diabetic mice and reduced the level of brain infarction back to that of control animal. Moreover, endothelial-specific HIF-1α knock-out mice were utilized to identify HIF-1's specific role in BBB disruption. HIF-1 inhibition ameliorated brain infarction and edema formation in diabetic animals after MCAO/reperfusion.
These results strongly indicate that HIF-1 plays an important role in hyperglycemia-aggravated BBB disruption in stroke and provide a novel therapeutic target for hyperglycemic stroke.

Disclosures: Z. Zhang: None. **H. Shi:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.18/Y8

Topic: C.09. Ischemia

Title: NRF-2 activation protects ischemic white matter injury

Authors: *S. K. AGRAWAL[1], V. KESHERWANI[2], F. ATIF[3], S. YUSUF[3];
[1]Univ. Nebraska Med. Ctr., OMAHA, NE; [2]Univ. of Nebraska medical Ctr., Omaha, NE;
[3]Emory Univ., Atlanta, GA

Abstract: Background: Oxidative stress damage plays a vital role in spinal ischemic injury. The nuclear factor erythroid 2-related factor (Nrf2) signaling pathway can be activated by cellular oxidative stress. Resveratrol, a plant-derived polyphenolic compound, has antioxidant property. Recent studies have demonstrated that resveratrol has protective effects against ischemic injury, however, its mechanism of action is not known. In the present study, we have studied neuroprotective effect of resveratrol and role of Nrf-2 in spinal cord ischemic injury.
Methods: Spinal cord was removed from adult male Wistar rats from T2-T10 and was used to induce ischemic injury in vitro with and without treatment with resveratrol (50μM). Electrophysiological recording of compound action potential (CAP) and oxidative stress was evaluated by biochemical assays. Pathological changes of spinal cord tissue were observed by Hemotxilin and Eosin (H & E) Staining and Western blot was used to quantify protein

expression levels of Glial Fribrillary Acidic Protein (GFAP) and Nrf2.
Results: We found significant changes in spinal cord white matter compound action potential (CAP) and H&E staining showed that resveratrol significantly improved neuronal injury. The biochemical assays showed significant changes in lipid peroxidase (LPO), glutathione peroxidase (GSH), superoxide dismutase (SOD), protein carbonyl (PC), mitochondrial energy (ATP) and mitochondrial Ca++. Further, we found significant increase in nuclear NrF-2 expression in nucleus with immunohistochemistry and western blotting and down regulation of GFAP content after resveratrol treatment as compared to injury.
Conclusion: The present results showed that resveratrol pretreatment had neuroprotective effects on compound action potential, Ca++ loading and biochemical parameters after ischemic injury. The neuroprotective effect is likely to be exerted by upregulated expression of transcription factor Nrf-2 to ameliorate the oxidative damage and preserving mitochondrial function.

Disclosures: S.K. Agrawal: None. **V. Kesherwani:** None. **F. Atif:** None. **S. Yusuf:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.19/Y9

Topic: C.09. Ischemia

Support: NIH Grant R01NS077521

Title: Cortical mapping of circuits affected by intracerebral hemorrhage in the mouse

Authors: *T. LANMAN, H. C. BARRATT, S. T. CARMICHAEL;
Neurol., David Geffen Sch. of Med., Los Angeles, CA

Abstract: Stroke is the fourth leading cause of death and the leading cause of adult disability in the United States. Intracerebral hemorrhage (ICH) represents a stroke type characterized by a high rate of mortality and disability. The processes of tissue repair and recovery following ICH have not been identified. ICH produces a sizeable region of cell death in the hemorrhage cavity. This study uses a retrograde neuronal tracer, Cholera Toxin Subunit B (CTb), to selectively label neurons projecting to the hemorrhage zone, which in the left striatum. The goal is to identify the cortical circuits that are affected, or disconnected, by the striatal damage from experimental ICH. Two models have been developed to simulate human ICH stroke in mice: Autologous Tail Blood Infusion (ATBI) and Collagenase Infusion (CI). Each models different aspects of ICH in humans. CTb was co-infused in conjunction with blood or collagenase with each model and with a saline control. This facilitates the comparison of the cortical distribution of neuronal

projections to the region of damaged striatum between hemorrhage and baseline in addition to comparing the distribution between the two ICH models. Labeled neurons were mapped with a computer-interfaced microscopic mapping program. Differences between stroke/baseline and between ATBI/CI were observed, thus indicating which areas of the cortex gained and lost neuronal links, or communication, with the damaged tissue following stroke. Preliminary study indicates differences in the ratio of contralesional projections to ipsilesional projections between stroke/baseline and ATBI/CI. The implications of these results will provide insight into the post-hemorrhagic stroke environment so that specific and effective treatments and therapies may be developed.

Disclosures: T. Lanman: None. **H.C. Barratt:** None. **S.T. Carmichael:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.20/Y10

Topic: C.09. Ischemia

Title: Tumor necrosis factor related apoptosis inducing ligand determines loss of the neuroprotective effect of preconditioning in rats undergone transient middle cerebral artery occlusion

Authors: *G. CANTARELLA[1], G. DI BENEDETTO[1], G. PIGNATARO[2], L. ANNUNZIATO[2], R. BERNARDINI[1];
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Abstract: The brain's resistance to ischemic injury can be transiently augmented by neuronal ischemic preconditioning (IPC), an endogenous neuroprotective modality by which a sub-lethal insult confers temporary protection against subsequent lethal ischemia. The preconditioning stimulus is recognized by molecular sensors, such as neurotransmitters, neuromodulators, cytokines and toll-like receptors, as well as ion channels and redox-sensitive enzymes. Cytokines are known to have an important role in acute stroke. A candidate protein involved in ischemic neuronal death is the Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL). We thus studied the role of TRAIL and its receptors in the pathophysiology of stroke and ischemic preconditioning (IPC) in rats subjected to transient middle cerebral artery occlusion (tMCAO). Data showed that TRAIL and its death receptors are up-regulated in cerebral ischemia, while

their down-regulation occurs when IPC is applied. Consistently, i.c.v. injection of TRAIL abrogates beneficial effects of IPC. Finally, to corroborate the hypothesis of a detrimental role of TRAIL in the ischemic rat brain, a TRAIL neutralizing antibody (CD253) was injected i.c.v. in rats subjected to tMCAO. The anti-TRAIL antibody exerted significant neuroprotective effects in rats subjected to tMCAO, with parallel amelioration of neurological deficits. All together, these data show that TRAIL is a deleterious factor in the pathogenesis of ischemia-related neuronal damage, and that the prevention of TRAIL effects in the brain may represent a novel neuroprotective strategy for treatment of stroke.

Disclosures: G. Cantarella: None. **G. Di Benedetto:** None. **G. Pignataro:** None. **L. Annunziato:** None. **R. Bernardini:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.21/Z1

Topic: C.22.Stroke Recovery

Support: NSC99-2320-B-006-027-MY3

Grant kmtth-101-004

Title: The evaluation of hyperbaric oxygen tratment in neurodegenerative diseases

Authors: C.-C. WU[1], I.-F. WANG[2,3], Y.-S. LEE[2], C.-P. SU[2], H.-K. WANG[2], I.-R. LO[4], *K.-J. J. TSAI[2,1];
[1]Inst. of Basic Med. Science, Natl. Cheng Kung Univ., Tainan, Taiwan; [2]Inst. of Clin. Medicine, Natl. Cheng Kung Univ., Natl. Cheng Kung University, Inst. of Clin. Med., Tainan, Taiwan; [3]Inst. of Life Science, Natl. Def. Med. Ctr., Taipei, Taiwan; [4]Inst. of Mol. Biology, Academia Sinica., Taipei, Taiwan

Abstract: The major brain diseases involve Alzheimer`s disease (AD), frontal temporal lobar degeneration (FTLD), and stroke…etc. More recent studies has focused on dementia with Aβ aggregations and TDP-43 inclusions, dementia after stroke and vascular contributions to cognitive decline and depression in later life, however, the efficient therapeutic strategies to cure these troublesome diseases are still in shortage. To date, several studies have provided evidence with regard to the neuroprotection benefits of hyperbaric oxygen (HBO) therapy in cases of trauma and brain disease, besides the improvements of bone marrow stem cells (BMSCs) proliferation and mobilization may also are involved in the therapeutic effects of HBO. Even

thought HBO has been used as a primary or adjunctive therapy over years, the course of therapeutic window is still controversial, whether long course HBO therapy improves a better prognosis is still an open question in brain diseases, in addition, the therapeutic mechanism of HBO-induced neuroprotection and functional recovery is still unexplored. We have generated the FTLD-U mouse model (CaMKII-TDP-43 transgenic mice) by overexpression of TDP-43 in the forebrain and the phenotypic characteristics mimicking those of FTLD. The middle cerebral artery occlusion (MCAO) rat stroke model is also applied on our researches. Hence, this study investigates the effects of HBO therapy on the migration of BMSCs, neurogenesis, gliosis, inflammation response and behavior performance. Our preliminary data indicated there was a better recovery of behavior performance in the three-week HBO group when compared with the two-day HBO group. Mobilization of BMSCs to the brain damaged area was more improved in long course HBO treatments, suggesting the duration of therapy is crucial for promoting the homing of BMSCs to injury brain by HBO therapies. HBO also can stimulate expression of trophic factors and improve neurogenesis and gliosis. These effects may help in neuronal repair after brain damage or degeneration, and increasing the course of HBO therapy might enhance therapeutic effects.

Disclosures: C. Wu: None. **I. Lo:** None. **K.J. Tsai:** None. **I. Wang:** None. **Y. Lee:** None. **C. Su:** None. **H. Wang:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.22/Z2

Topic: C.09. Ischemia

Title: Time-dependent formation/elimination of nucleic γ-H2AX and its cytosolic distribution in a rat hippocampal neuron culture model of ischemic stroke

Authors: *A. I. MARTIN[1], R. D. SWEAZEY[1], B. C. HONG-GOKA[2,1], F.-L. F. CHANG[1]; [1]Indiana Univ. Sch. of Med. - Fort Wayne, Fort Wayne, IN; [2]UCSF - Fresno Alzheimer's & Memory Ctr., Fresno, CA

Abstract: Stroke is a major health issue often resulting in death or disability. Many factors contribute to the cellular damage resulting from the complex and multistage processes of hypoxic ischemia. The precise biochemical events that account for the death of hippocampal neurons either by apoptosis or necrosis during hypoxia remain poorly understood. While delayed cell death in the brain under ischemic conditions has been documented in humans and

experimental models, the early molecular events occurring before reperfusion and determining the extent of cellular loss are less well understood. Thus, we investigated the time course of γ-H2AX formation and elimination in an oxygen and glucose deprivation (OGD) model of ischemia in primary rat postnatal hippocampal neuron-rich culture. γ-H2AX is the phosphorylated variant of the H2A protein family, which is a component of the histone octomer in nucleosomes and is phosphorylated by kinases as the first step in recruiting and localizing DNA repair proteins. It has been used effectively as a marker for DNA damage detecting DNA double-strand breaks (DSBs) in tissues from patients with chronic obstructive pulmonary disease, solid tumors, or myeloid leukemia.
Using fluorescent immunocytochemical techniques, our results show that H2AX becomes increasingly phosphorylated with increasing OGD duration, peaking between 2 and 4 hours of OGD and reversing to normoxic levels at 8 hours OGD. In agreement with published reports, bright nuclear foci and diffuse nuclear staining were seen in a time-dependent fashion in apoptotic cells depicting the nuclear accumulation of γ-H2AX at the DNA damage sites. Extended durations of OGD produced γ-H2AX negative cells that were characterized as late-apoptotic or undergoing DNA repair. In addition, we also observed a more diffuse staining in the cytoplasm and axon hillock of some cells exposed to OGD, suggesting a possible pre-apoptotic modification of H2AX. Investigations of these different γ-H2AX staining patterns (nucleic vs. cytosolic) and underlying mechanisms are ongoing. We believe that γ-H2AX, a well-documented early, sensitive, and selective marker of DNA double-strand breaks, is useful in the identification and quantification of ischemic neurons that are compromised, yet salvageable and that it provides a unique opportunity to test potential new treatments for hypoxic ischemia.

Disclosures: A.I. Martin: None. **R.D. Sweazey:** None. **B.C. Hong-Goka:** None. **F.F. Chang:** None.

Poster

537. **Ischemia: Pathophysiology, Biomarkers and Treatment**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 537.23/Z3

Topic: G.04. Physiological Methods

Title: Quantifying hemodynamic changes in a transient filament model of focal cerebral ischemia with 2d optical imaging spectroscopy

Authors: *F. BURROWS[1], A. DENES[2], N. BRAY[2], S. ALLAN[2], I. SCHIESSL[2];
[1]Univ. Of Manchester, Manchester, United Kingdom; [2]Univ. of Manchester, Manchester, United Kingdom

Abstract: The aim of this study is to develop a novel model of transient cerebral ischaemia for functional brain imaging with high spatial and temporal resolution. Furthermore we developed spectroscopic mulitwavelength analysis to quantify the dynamic changes in cortical blood perfusion before, during and after induced cerebral ischaemia in anesthetized mice.
Middle cerebral artery occlusion (MCAo) was induced by advancing a novel remote filament along the internal carotid artery to occlude the middle cerebral artery. Using the remote filament it is possible to gain baseline recordings of the cerebral vasculature before induced stroke and subsequent recordings during and after for upto 6 hours of reperfusion.
2D optical imaging spectroscopy with four different wavelengths of illumination allows the calculation of micromolar changes in the concentration of heamodynamic parameters; oxy-, deoxy- and total haemoglobin.
Our results show the expected decrease in the concentration of oxyhaemoglobin in the occluded MCA and supplying cortical regions compared to baseline. After removal of the filament, the MCA shows an increase in oxyhaemoglobin concentration even though large areas of the cortex which are supplied by this vessel are insufficiently reperfused even several hours after the stroke. These findings support the idea of a 'no-reflow' phenomenum.
Our results suggest that 2D optical imaging spectroscopy with the novel remote filament approach enables precise mapping of vascular and metabolic changes in the mouse MCAo model. Obtaining baseline recordings makes this a powerful model with multiple applications contributing to better understanding of ischaemic-induced changes in the brain

Disclosures: F. Burrows: None. **A. Denes:** None. **N. Bray:** None. **S. Allan:** None. **I. Schiessl:** None.**Poster**

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.01/Z4

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NINDS Grant NS065343

NIH/NINDS Grant NS059962

NIH/NCRR Grant P30GM103400

Title: Role of AMPA receptors in homocysteine-NMDA receptor mediated crosstalk between ERK and p38 MAP kinase

Authors: *R. PODDAR, S. PAUL;
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Abstract: Hyperhomocysteinemia or systemic elevation of homocysteine is considered to be a major risk factor for neurodegenerative disorders but little is known about the underlying mechanisms involved in homocysteine mediated neuronal cell death. Since homocysteine is a known agonist of N-methyl-D-aspartate receptors (NMDAR), it had been assumed that homocysteine activates NMDA-mediated intracellular pathways in ways analogous to the prototype NMDAR agonist, glutamate. However, recently we have shown that the effects of homocysteine and glutamate on NMDAR activation and subsequent downstream signaling are quite different. While glutamate-mediated neuronal death involves activation of NR2B subunit containing NMDAR (NR2B-NMDAR), our findings showed that homocysteine-induced neuronal death involves stimulation of NR2A subunit containing NMDAR (NR2A-NMDAR) that are generally thought to be involved in neuronal survival. Our findings also showed that glutamate-NMDAR stimulation leads to a transient activation of ERK MAPK (extracellular-signal regulated mitogen activated protein kinase) that plays a role in neuronal survival. In contrast, homocysteine-NR2A NMDAR mediated activation of ERK MAPK is sustained, which leads to neuronal cell death. More recently, we showed that stress-induced p38 MAPK also plays a role in homocysteine-NMDAR induced neuronal death. We further established that homocysteine-mediated neurotoxicity involves a novel interplay between ERK and p38 MAPK, where activation of p38 MAPK is downstream of, and dependent on ERK MAPK.
The goal of the present study is to evaluate the underlying signaling mechanism(s) involved in homocysteine-mediated crosstalk between ERK and p38 MAPK. Using cortical neuronal cultures we show that inhibition of amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPAR) with CNQX blocks homocysteine-mediated phosphorylation of p38 MAPK but had no effect on ERK MAPK phosphorylation suggesting that AMPAR plays an intermediary role in facilitating the crosstalk between ERK and p38 MAPK. Using surface biotinylation studies we further show that treatment with homocysteine leads to internalization of GluR2-AMPAR subunits from cell surface. ERK MAPK inhibitor PD98059 attenuates this effect of homocysteine on GluR2-AMPAR. Consistent with these findings inhibition of GluR2-lacking AMPARs with NASPM significantly reduces p38 MAPK phosphorylation and subsequent cell death. These findings suggest that homocysteine-ERK MAPK dependent decrease in surface GluR2-AMPAR increases Ca2+ influx through GluR2-lacking AMPAR resulting in activation of p38 MAPK and subsequent neuronal cell death.

Disclosures: R. Poddar: None. **S. Paul:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.02/Z5

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH MH091407

VA Center of Excellence for Stress and Mental Health

VISN 22 Mental Illness Research, Education, and Clinical Center

Title: Intracerebroventricular administration of interleukin-1β elevates brain kynurenic acid and disrupts PPI in C57BL/6 mice

Authors: S. CALDWELL[1], M. LARSSON[2], M. KAMENSKI[1], L. SCHWIELER[2], G. ENGBERG[2], V. B. RISBROUGH[1,3], S. ERHARDT[2], *S. B. POWELL[1,3];
[1]UCSD, LA JOLLA, CA; [2]Physiol. and Pharmacol., Karolinska Institutet, Stockholm, Sweden; [3]VA Ctr. of Excellence for Stress and Mental Hlth., La Jolla, CA

Abstract: *Background*: Interleukin-1 beta (IL-1β) is a cytokine protein (member of the interleukin 1 cytokine family) and an important mediator of the inflammatory response. Patients with schizophrenia and bipolar disorder, particularly those that have had a psychotic episode, present elevated levels of IL-1β and kynurenic acid (KYNA). KYNA is produced via the kynurenine pathway of tryptophan metabolism, and immune activation can shift tryptophan metabolism from 5-HT to l-kynurenine. In rodents, pharmacologically elevated levels of KYNA have been shown to disrupt prepulse inhibition of startle (PPI). The aim of the present study was to investigate whether IL-1β influences the synthesis of brain KYNA in mice and if administration of IL-1β affects PPI. *Methods*: IL-1β was injected intracerebroventricularly (ICV) at doses 0.5, 5 and 50 ng. Mice were tested in startle and PPI every hour for 6 hours. Weight and temperature of the mice were also recorded every hour. Another set of C57BL/6 mice were injected ICV with 0.5, 1, 5, or 10 ng of IL-1β and the animals were euthanized and brain KYNA was quantified by means of HPLC 6 hours post-injection. *Results*: ICV administration of IL-1β disrupted PPI at the lowest dose tested (0.5 ng; $p<0.05$). Higher dose of IL-1β decreased startle magnitude. Administration of 0.5 ng IL-1β, but not 1, 5, or 10 ng, significantly elevated brain KYNA levels compared to vehicle (9.49 ± 1.88 nM *vs.* 3.40 ± 0.51 nM , $p < 0.05$). *Discussion*: Notably, only administration of the lowest dose of IL-1β disrupted PPI, indicating that this effect may be mediated by the increased brain KYNA concentrations observed at this dose. These data support the hypothesis that IL-1β and KYNA are important players in the pathophysiology of psychotic diseases such as schizophrenia and bipolar disorder.

Disclosures: S. Caldwell: None. **M. Larsson:** None. **M. Kamenski:** None. **L. Schwieler:** None. **G. Engberg:** None. **V.B. Risbrough:** None. **S. Erhardt:** None. **S.B. Powell:** A. Employment/Salary (full or part-time):; University of California San Diego. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Servier.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.03/Z6

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: Research Grant Programme of the European Society of Anaesthesiology

Thorsten Söderberg Foundation, Sweden

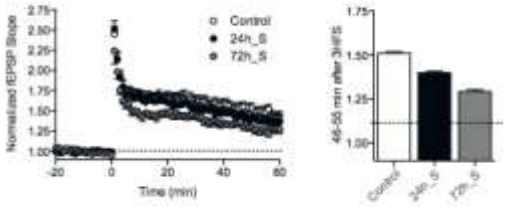
Research Council for Medicine, Sweden

Title: Surgery affects synaptic plasticity and astrocyte activity contributing to postoperative cognitive decline

Authors: M. GÓMEZ[1], T. YANG[1], M. LINDSKOG[2], L. ERIKSSON[1], *N. TERRANDO[1]; [2]Neurosci., [1]Karolinska Institutet, Stockholm, Sweden

Abstract: Background: Recovery from major surgery or critical illness is often accompanied by disturbances in cognitive domains and is a major clinical problem of unclear etiology. Activation of the innate immunity, pro-inflammatory cytokine release and neuroinflammation are putative mechanisms underlying cognitive dysfunction in preclinical surgical models, yet the mechanisms whereby systemic injury affects CNS function remain unclear. Herein we explore the effects of surgery on synaptic plasticity and neuroinflammation following orthopedic surgery under general anesthesia.
Methods: We used 11-14-wk-old male wildtype (WT) C57BL/6 and randomly assigned to untreated control animals with analgesia or surgery (an open tibial fracture of the left hind leg with intramedullary fixation) under isoflurane general anesthesia and postoperative analgesia. Separate cohorts of animals were used to assess synaptic plasticity, systemic and central inflammatory changes including astrocytes (GFAP) immunofluorescence, and hippocampal-dependent cognition using trace fear conditioning (TFC).
Results: Aside a key role of myeloid-derived peripheral macrophages in penetrating the blood-brain barrier after surgery, we now found distinct changes in astrocytes activation and morphology. Reactive astrocytes, with enlarged cell bodies and reduced filaments, were observed 24h after surgery. Astrocytic alteration was further associated with impaired pre-synaptic facilitation and long-term potentiation (LTP) deficit starting at 24h and further decreasing 72h postoperatively (figure 1). At 72h, surgical animals were also tested with TFC to assess hippocampal-dependent memory function and further displayed memory dysfunction ($P<0.001$).

Conclusion: Peripheral surgery affects synaptic transmission and plasticity causing postoperative cognitive decline. Neuroinflammation, including astrocyte activation, may be pathological hallmarks in surgery-induced cognitive decline.



Disclosures: M. Gómez: None. **T. Yang:** None. **M. Lindskog:** None. **L. Eriksson:** None. **N. Terrando:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.04/Z7

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: AHA 09BGIA2080137

NIH K01AG031926

NIH R01AT007317

NIH R01NS078026

Title: Misoprostol protects brain against intracerebral hemorrhage

Authors: *J. WANG, H. WU, T. WU, X. ZHAO, W. CHEN;
Anesthesiology/Critical Care Med., Johns Hopkins Univ., Sch. of Med., BALTIMORE, MD

Abstract: Intracerebral hemorrhage (ICH) is a devastating form of stroke that leads to significant disability in survivors. Misoprostol, a synthetic PGE_1 analog and PGE_2 receptor agonist, has shown protection against cerebral ischemia. In this study, we tested the efficacy of misoprostol in 12-month-old mice subjected to collagenase-induced ICH. We also investigated its potential mechanism of action. In the present study, ICH was induced by intrastriatal injection

of collagenase, and mice were randomly assigned to receive subcutaneous injections of misoprostol or vehicle. Neurologic deficits were examined on days 3 and 28 after ICH. Some mice were sacrificed after 1 day for measurement of superoxide production, protein oxidation, gelatinolytic activity, HMGB1 expression, Src kinase activity, and cytokine interleukin-1β expression. Others were sacrificed on day 3 for measurement of brain injury volume, swelling, edema, neuronal death, and cellular inflammatory response. Misoprostol post-treatment decreased brain swelling and lesion volume and improved long-term neurologic function. However, misoprostol significantly increased the incidence of diarrhea without significantly increasing body weight loss or mortality. Misoprostol post-treatment decreased cellular inflammatory response, including microglia/macrophage and astrocyte activation and neutrophil infiltration; attenuated oxidative brain damage and gelatinolytic activity; and decreased HMGB1 expression, Src kinase activity, and interleukin-1β expression without affecting cyclooxygenase-2 expression. These results indicate that misoprostol protects brain against ICH injury through mechanisms that may involve the HMGB1, Src kinase, and MMP-2/9 pathway; however, the high incidence of diarrhea could be a concern for its clinical use.

Disclosures: J. Wang: None. **H. Wu:** None. **T. Wu:** None. **X. Zhao:** None. **W. Chen:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.05/Z8

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Plasma levels of neuron specific enolase quantifies the extent of neuronal injury in murine models of ischemic stroke and multiple sclerosis

Authors: *T. DAEHN[1], M. GELDERBLOM[1], B. SCHATTLING[2], P. LUDEWIG[1], C. BERNREUTHER[1], P. ARUNACHALAM[1], M. GLATZEL[1], C. GERLOFF[1], M. A. FRIESE[2], T. MAGNUS[1];
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Abstract: Objective: We aimed at validating a plasma biomarker for neuronal damage that can be used in acute and chronic models of neurological diseases.
Methods: We investigated two different models, middle cerebral artery occlusion followed by reperfusion and MOG35-55-induced experimental autoimmune encephalomyelitis (EAE). In stroke experiments we measured infarct sizes by magnetic resonance imaging and vital stainings

and correlated them with plasma levels of neuron specific enolase (NSE) at different time points after reperfusion. Equally, in EAE experiments, we correlated NSE levels with neurological scores and histopathological damage of axons at different time points. We detected plasma NSE levels by ELISA.
Results: Plasma NSE levels correlated significantly with stroke size and EAE score. Investigations into the dynamics of neuronal loss over time correlated well with the dynamics of NSE levels. NSE even predicted the onset of EAE, before clinical signs were recordable.
Conclusions: Plasma NSE is a valid and simple experimental biomarker that allows quantifying the degree of neuronal injury in a non-invasive approach.

Disclosures: T. Daehn: None. **M. Gelderblom:** None. **T. Magnus:** None. **P. Ludewig:** None. **C. Bernreuther:** None. **P. Arunachalam:** None. **M. Glatzel:** None. **C. Gerloff:** None. **M.A. Friese:** None. **B. Schattling:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.06/Z9

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Selective P2X7 receptor antagonists inhibit Bz-ATP induced IL-1β release in the rat brain

Authors: *I. FRASER, L. ALUISIO, P. BONAVENTURE, B. SAVALL, M. LETAVIC, N. CARRUTHERS, T. LOVENBERG, A. BHATTACHARYA;
Janssen, SAN DIEGO, CA

Abstract: The P2X7 receptor plays an important role in the release of pro-inflammatory cytokines (IL-1β, IL-18) from cells of the immune system in both the periphery and the central nervous system. It has been demonstrated that extracellular release of mature IL-1β is dependent on ATP-sensitive P2X7 activation. IL-1β released from glial cells may play a role in initiating the neuroinflammatory cascade in the brain, possibly leading to various neuropsychiatric and neurodegenerative disorders. Therefore, the P2X7 receptor has become an attractive drug target for the treatment of diseases that involve neuroimmune processes. Here we report on a new dialysis method to detect IL-1β directly from the hippocampus of freely moving rodents. To validate this new method, we tested the effect of the P2X7 agonist dibenzoyl-ATP (Bz-ATP) in wild type (WT) and P2X7 knockout (KO) mice. Additionally, three brain penetrant P2X7 antagonists, Compound A (2-Methyl-N-{[1-(4-phenylpiperazin-1-yl)cyclohexyl]methyl}-1,2,3,4-tetrahydroisoquinoline-5-carboxamide), Compound B (N-{[4-(4-Phenylpiperazin-1-

yl)tetrahydro-2H-pyran-4-yl]methyl}-2-(phenylsulfanyl)pyridine-3-carboxamide) and Compound C were tested on Bz-ATP-induced IL-1β release in the rat hippocampus. Bz-ATP was found to increase IL-1β release in the hippocampus of WT but not P2X7 KO mice. The three structurally distinct P2X7R antagonists inhibited Bz-ATP induced IL-1β release in the rat hippocampus. Taken together these data show evidence that treatment with a P2X7 receptor antagonist may modulate pro-inflammatory cytokines involved in neuroinflammatory diseases.

Disclosures: I. Fraser: A. Employment/Salary (full or part-time):; Jassen. **L. Aluisio:** A. Employment/Salary (full or part-time):; Jassen. **P. Bonaventure:** A. Employment/Salary (full or part-time):; Jassen. **B. Savall:** A. Employment/Salary (full or part-time):; Jassen. **M. Letavic:** A. Employment/Salary (full or part-time):; Jassen. **N. Carruthers:** A. Employment/Salary (full or part-time):; Jassen. **T. Lovenberg:** A. Employment/Salary (full or part-time):; Jassen. **A. Bhattacharya:** A. Employment/Salary (full or part-time):; Jassen.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.07/Z10

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH DA023085

Title: Single high dose methamphetamine recruits dopamine transporter and parkin to rat striatal terminals in a microtubule dependent manner

Authors: *B. A. KILLINGER, A. MOSZCZYNSKA;
Dept. of Pharmaceut. Sciences, EACPHS, Wayne State Univ., Detroit, MI

Abstract:
Methamphetamine (METH) is a potent psychostimulant that can cause toxicity to both dopaminergic (DAergic) and serotonergic (5HTergic) nerve terminals in the striatum. METH inhibits and reverses the function of dopamine transporter (DAT); the membrane bound protein responsible for the reuptake of dopamine (DA) from the synapse. The regulation of DA influx/efflux across the presynaptic terminal of DAergic terminals via DAT is the primary factor mediating METH neurotoxicity. The efficient trafficking of membrane-bound proteins to their cellular targets is required for the protein to retain proper functioning. However, the regulation of DAT protein trafficking in response to METH is still poorly understood. In vitro, METH causes a transient recruitment of DAT to the presynaptic membrane, but in vivo METH administration does not effect localization of DAT in the presynaptic nerve terminals. In cultured cells, the

interaction of E3 ligase parkin with DAT inhibits the activity of DAT at the presynaptic membrane and the disruption of microtubule polymerization increases membrane expression of DAT. Previously, our lab have found that acute administration of METH quickly increases total levels of both parkin and DAT in the rat striatum suggesting that METH quickly increases DAT trafficking in a parkin dependent manner. The current study investigated whether this METH induced recruitment of DAT via parkin was dependent upon microtubule polymerization. We found that intracerebroventricular injection of the microtubule polymerization inhibitor, colchicine, increased the membrane expression of DAT. Furthermore pre-treatment with colchicine prior to IV METH inhibited the increase in DAT levels in the striatum. Our results suggest that, in vivo, the METH induced trafficking of DAT to the striatal presynaptic membrane depends upon both its interaction with parkin and the polymerized microtubules. This further supports the in vivo role of DAT trafficking in the early cellular response to METH.

Disclosures: B.A. Killinger: None. **A. Moszczynska:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.08/Z11

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R15 NS060105

Title: Lipocalin-2 induction in sterile neuroinflammation: A marker for neuronal cell death

Authors: M. BANJARA[1], K. BENNETT[3], A. FERNANDEZ[2], *J. STOLL[4];
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Abstract: Lipocalin-2 (LCN2) is a bacteriostatic protein that sequesters siderophore-bound iron and is induced by lipopolysaccharide in C6 astroglial cells and cultured astrocytes. LCN2 is also induced in aseptic neuroinflammation. However, the role in sterile neuroinflammation is unknown and we undertook studies to understand its function. An in vitro model was developed where cells were treated necrotic cell extract and LCN2 induction was measured by quantitative RT-PCR and western blot. We also measured the induction of LCN2 in vivo in two mouse epilepsy models: pilocarpine-induced status epilepticus (SE) or corneal kindling. LCN2 was induced in hippocampus of SE mice, but not in corneal kindled mice. Induction in SE mice was maximal at 3 days post-SE, corresponding to the day of maximum cell death. Induced LCN2

declined to basal levels by 21 days post-SE along with cell death. Kindled mice did not show hippocampal cell death at any time. These data suggest LCN2 may be a marker of pathological cell death. It is believed that damage-associated molecular patterns, released by necrotic cells, alert the innate immune system during inflammatory insults. Thus, we decided to identify the molecule(s) from dead cells that trigger LCN2 expression. C6 cells treated with 300µg/ml necrotic Neuro2A cell extract for 18-24 hrs induced LCN2 mRNA from 5- to 550-fold in several different preparations. Necrotic cell extract did not induce LCN2 expression in cultured astrocytes. However, basal LCN2 was high in primary astrocytes perhaps due to exposure to dead cells during culture preparation. In fact, the supernate following centrifugation of isolated astrocytes induced LCN2 expression in C6 cells. LCN2 induction in C6 cells was blocked by NF-κB nuclear translocation inhibitor, Bay11-7082. The LCN2 promoter contains a NF-κB response element and this may explain the induction of LCN2 mRNA. Induction was inhibited 47% by FPS-ZM1, a RAGE receptor antagonist but not by IL-1RA. VIPER, a peptide that inhibits TLR4 signal transduction inhibited LCN2 mRNA induction by 27%. These data suggest multiple proteins in the extract are responsible for induction. However, a cytokine array failed to show elevated cytokines, ruling out their role. The necrotic extract was fractionated by gel filtration chromatography as a step to identify the responsible molecules. However, every fraction eluting from the column showed inducing activity, thus confirming that multiple proteins are involved. We propose, based on the diversity of the inducer proteins, that the induction is due to non-specific structural alterations of necrotic proteins rather than the release of specific inducer proteins.

Disclosures: M. Banjara: None. **K. Bennett:** None. **A. Fernandez:** None. **J. Stoll:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.09/Z12

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR

Title: Emotional and cognitive behaviour changes are associated with increased corticosterone and changes in glutamatergic transmission in the early stages of experimental allergic encephalomyelitis (EAE), a mouse model of multiple sclerosis

Authors: *S. ACHARJEE[1], N. NAYANI[1], M. TSUTSUI[1], M. N. HILL[2], S. S. OUSMAN[3], Q. J. PITTMAN[1];

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Abstract: Multiple sclerosis (MS) is often associated with co-morbid neuropsychiatric and cognitive impairments, affecting around 50% of MS patients. Herein, we investigated these abnormalities in an animal model of MS, called EAE, during the presymptomatic stage of the disease.
EAE was induced by immunization with MOG35-55 and the mice exhibited no motor deficits until d9 after immunization. This enabled us to carry out a series of neurobehavioral tests between d6-d8 post-immunization. EAE mice spent more time in the outer zone in open field test and in the closed arms of elevated plus maze, and showed decreased latency for immobility in tail suspension test and forced swim test compared with controls, which were indicative of anxiety- and depression- like behavior. EAE mice spent less time in the target quadrant compared to sham controls during probe trial in Morris water maze while in fear conditioning test, it displayed a trend towards faster memory extinction, indicative of memory impairment. No demyelination, microglial activation or astrogliosis was observed in the brain at this stage. Transcript analysis by RT-PCR from the brain revealed elevated IL-1β and TNF-α in the hypothalamus of EAE mice. This was associated with increased plasma corticosterone levels in EAE mice compared to control. To investigate the neuronal correlate of these behaviour changes, whole-cell recording was carried out in the principal neurons of the basolateral amygdala. There was no difference in the membrane excitability (action potential threshold and firing rate) between control and EAE mice. Investigation into glutamatergic synaptic transmission revealed increased frequency of the mini-excitatory postsynaptic currents (mEPSC) without any changes in amplitude compared to the controls; however, the paired pulse ratio of the evoked EPSCs was increased implying decreased release probability.
In conclusion, emotional and cognitive deficits are observed in EAE (and possibly MS) in the absence of demyelination and was associated with inflammatory and HPA axis changes and changes in glutamatergic synapses.

Disclosures: S. Acharjee: None. **N. Nayani:** None. **M. Tsutsui:** None. **M.N. Hill:** None. **S.S. Ousman:** None. **Q.J. Pittman:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.10/Z13

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: 1IK2BX001686-01

Title: Determining the mechanism of orexin A induced neuroprotection in an *Ex vivi* arcuate nucleus model

Authors: *C. M. DUFFY[1], C. J. BILLINGTON[5,2], C. M. KOTZ[5,3,4], J. P. NIXON[5,4], T. A. BUTTERICK[5,4];
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Abstract: Diets rich in saturated fatty acids such as palmitic acid increase oxidative stress, apoptosis, and pro-inflammatory cytokines in both peripheral tissue and the central nervous system, including the cerebral cortex and the hypothalamus. High fat diets have also been shown to induce neuronal degeneration of sites important to regulating energy balance, such the arcuate nucleus, but the underlying cause of this dysregulation remains unclear. In cell culture models, palmitic acid increases lipid peroxidation and increases cell death, and in an in vitro adult mouse hypothalamic cell culture model, we showed that orexin A (hypocretin) reduces lipid peroxidation, decreases caspase 3/7 activity induced by palmitic acid and stabilizes B-cell lymphoma-2 (Bcl-2). These data suggest that orexin A may be neuroprotective, but the mechanism underlying this effect is unknown. We hypothesized that a potential mechanism could be gene expression stabilization of the anti-apoptotic protein Bcl-2. To test this possibility, we used an organotypic “ex vivo” brain model, using a micropunch of the arcuate nucleus which allowed for pharmacological manipulation while preserving the functional integrity of the arcuate nucleus network. Arcuate nucleus tissue explants were isolated from Sprague-Dawley rat brains, and exposed to either palmitic acid (0.075 mM) or control solution. Following 24 hour of palmitic acid exposure (or not), cell viability was tested using a resazurin-based assay. In the palmitic acid treated explants, there was a significant decrease in cell viability ($p < 0.05$) compared to controls, suggesting the stabilization of Bcl-2. We are currently evaluating mechanisms of orexin A-induced neuroprotection in arcuate nucleus explants following chronic palmitic acid treatment in the presence or absence of OxA, and measuring changes in pro- and anti-apoptotic gene expression such as Bcl-2 family members. Based on cell culture models, we hypothesize that OxA will increase viability of Arc explants during PA challenge. Collectively, this data will be used to gain further insight into mechanisms of OxA-induced neuroprotection.

Disclosures: C.M. Duffy: None. **T.A. Butterick:** None. **C.J. Billington:** None. **C.M. Kotz:** None. **J.P. Nixon:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.11/Z14

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AG020569

NIH Grant AG028367

Title: Complex response of brain endothelial cells to hypoxia: Implications for Alzheimer's disease

Authors: J. LUO, A. PANDEY, X. YIN, *J. M. MARTINEZ, P. GRAMMAS;
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Abstract: Hypoxia is increasingly recognized as an important contributing factor to the development of brain diseases such as Alzheimer's disease (AD). Although hypoxia is a powerful regulator of angiogenesis in the periphery the response of brain endothelial cells to hypoxic challenge appears to be unique. We have previously demonstrated that exposure of brain endothelial cell cultures to hypoxia resulted in a significant decrease in tube length, an in vitro index of angiogenesis, despite increases in several pro-angiogenic proteins. The objective of this study was to further explore the biochemical processes that regulate the response of brain endothelial cells to hypoxia. Brain endothelial cell cultures were initiated from isolated rat brain microvessels and subjected to hypoxia (1% O2) for various time periods. The results showed that hypoxia induced rapid ($\leq$ 0.5h) expression of hypoxia-inducible factor 1α (HIF-1α) and that cell viability, assessed by MTT assay, was unaffected within the first 8 h. While hypoxic exposure of brain endothelial cell cultures evoked a decrease in tube length (angiogenesis) it did not induce apoptosis. On the contrary, apoptosis induced by nutrient deprivation in these cultures was mitigated by hypoxia. Treatment of endothelial cell cultures with resveratrol evoked an increase in apoptotic signaling as indicated by expression of apoptosis-related proteins, cleaved caspase 3, Bim 1 and phospho-p53. The blunted angiogenic response detected in hypoxic cultures was partially reversed with reveratrol pretreatment, implicating apoptotic signaling in angiogenesis. The idea that there are overlapping or common mechanisms in apoptosis and angiogenesis in brain endothelial cells is supported by data which showed that brain endothelial cells grown under normoxic conditions and exposed to the apoptosis inhibitor pifithrin (30 μM) demonstrated impaired angiogenesis. Taken together these data implicate a complex relationship in the cerebral microcirculation among hypoxia, apoptosis and angiogenesis and suggest that targeting mechanisms and/or mediators that drive endothelial activation toward angiogenesis may be useful in AD and other diseases characterized by cerebral hypoxia.

Disclosures: J. Luo: None. **A. Pandey:** None. **X. Yin:** None. **J.M. Martinez:** None. **P. Grammas:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.12/Z15

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: ViCTOR supplement to R01ES03299

Title: Chronic low-dose methylmercury treatment disrupts mitochondrial ATP production of striatal synaptosomes in male BALB/c mice

Authors: *S. M. FOX, W. D. ATCHISON;
Dept. of Pharmacol. and Toxicology, Michigan State Univ., East Lansing, MI

Abstract: Methylmercury (MeHg) is an environmental contaminant that causes cell-type specific damage to the central nervous system in a calcium (Ca^{2+}) dependent manner. *In vitro* acute application of MeHg results in a time- and concentration-dependent biphasic increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$). Treatment with voltage-gated calcium channel (VGCC) antagonists delay the MeHg-induced Ca^{2+}-increase. MeHg targets mitochondrial regulation by causing decreased Ca^{2+} uptake, increased Ca^{2+} release, opening of the mitochondrial permeability transition pore, and disruption of oxidative phosphorylation. The acute effects of MeHg have been investigated extensively however the effects of chronic low-dose exposure have not been characterized. The objective of this study was to investigate the effects of an *in vivo* chronic low-dose MeHg treatment on mitochondrial function of nigrostriatal dopamine (NSDA) neuron synaptosomes and whether co-treatment with the VGCC antagonist, isradipine, would prevent MeHg-induced changes. The NSDA cell bodies are located in the midbrain and they project their axons to the striatum. These neurons exhibit a unique physiological phenotype; they autonomously generate action potentials in the absence of synaptic input. The spontaneous action potentials rely on the influx of Ca^{2+} through Cav1.3, L-type Ca^{2+} channels. Male Balb/c mice were given free access to 0 or 6.25 ppm Hg as MeHg in their drinking water and 0 or 2 ppm isradipine in their feed. Following a 12mo treatment striatal synaptosome mitochondrial basal respiration, ATP production, maximal respiration, and spare capacity were measured using an Extracellular Flux Analyzer. MeHg treatment alone tended to decrease ATP production. Co-treatment with the VGCC antagonist isradipine resulted in a significant increase in ATP production compared to isradipine or MeHg treatment alone. Treatment had no effect on basal

respiration, maximum respiration, or spare capacity. These data suggest chronic low-dose MeHg treatment induces mitochondrial dysfunction resulting in decreased ATP production in striatal synaptosomes. Isradipine co-treatment was able to prevent the MeHg-induced decrease in ATP production suggesting that MeHg-induced damage is dependent on functional VGCC's in this chronic treatment paradigm. This work was supported by a ViCTOR supplement to R01ES03299.

Disclosures: S.M. Fox: None. **W.D. Atchison:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.13/Z16

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: PAEP, UNAM

Title: Inflammatory changes in brain after ozone exposure

Authors: *E. G. GUEVARA[1,2], J. MARTÍNEZ-LAZCANO[1], V. CUSTODIO RAMÍREZ[1], M. HERNÁNDEZ-CERÓN[1,3], C. RUBIO OSORNIO[1], C. PAZ TRES[1];
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Abstract: Ozone (O_3), a component of air pollution has a significant impact on public health. The O_3 is a highly reactive gas that oxidizes molecules in the biological systems and reacts with cell membranes inducing the formation of reactive oxygen species that produce the accumulation of oxide derivates and inflammatory processes in airway epithelial cells. Central nervous system (CNS) also has been affected, experimentally induce changes in sleep patterns and neurotransmitters related to sleep following O_3 exposure these changes were revert by anti-inflammatory drug administration. In the present study we analyze the airway inflammatory response produced by O_3 exposure and its consequences in the CNS in both acute and chronic exposure. We use the ELISA technique to determine TNF-α concentrations and western blot technique to quantify GFAP protein expression.
We found a significant increase of TNF-α in the lungs after acute 1 ppm O_3 exposure, high levels remain after chronic O_3 exposure. While in brain, both the TNF-α and GFAP increase since the acute exposition and remain until chronic O_3 exposure. The cytokine TNF-α has been related to changes in the sleep pattern and related neurotransmitters in sleep studies and the GFAP is

considered a marker of activated glial cells during brain inflammation. Then, we postulate that such sleep changes produced by O_3 exposure could be due to the inflammatory response of the CNS.

Disclosures: E.G. Guevara: None. **J. Martínez-Lazcano:** None. **V. Custodio Ramírez:** None. **M. Hernández-Cerón:** None. **C. Rubio Osornio:** None. **C. Paz Tres:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.14/Z17

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Inhibition of nitrodative-inflammatory cascade in experimental paradigm of quinolinic acid -induced neurotoxicity: Neuroprotective profile of sesamol and quercetin

Authors: *A. KUHAD, S. SINGLA, V. ARORA, K. CHOPRA;
Panjab Univ., CHANDIGARH, India

Abstract: Quinolinic acid (QA), a well known excitotoxin that produces a pharmacological model of Huntington's disease in rats and primates, has been shown to evoke degenerative events in nerve tissue via NMDA receptor overactivation and oxidative stress to exert its neurotoxic actions. Thus the affiliation of QA induced oxidative stress induced neuroinflammtory cascade represents a rational to investigate the neuroprotective role of naturally occuring antioxidants like sesamol and quercetin in the experimental paradigm of Huntington's disease. Rats were intrastriatally administered quinolinic acid and were treated with sesamol (4, 8 and 16 mg/kg, i.p) and quercetin (25, 50 and 100 mg/kg, i.p) for 14 days before and 14 days after quinolinic acid administration, with these natural antioxidants. Results demonstrated that intrastriatal injection of QA leads to increased escape latency, impaired locomotor activity, as well as significant increase in immobility time in forced swim test, This behavioural deficit was integrated with the increased nitrodative stress markers (increased lipid peroxidation, raised nitrite concentration and depletion of endogenous antioxidants such as catalase, superoxide dismutase and reduced glutathione) along with the significant increase in the TNF-α levels in rat brain suggesting QA mediated oxidative and neuroinflammatory damage. More over intrastriatal administration of QA resulted in significant decrease in the levels of dopamine, serotonin and norepinephrine in the rat forebrain. Chronic treatment with sesamol (4, 8 and 16 mg/kg, i.p) and quercetin (25, 50 and 100 mg/kg, i.p) attenuated these behavioral, biochemical and neurochemical alterations in the rat brain and these effects were attributed to their strong

antioxidant and anti-inflammatory potential. Conclusively it is suggested that major features of QUIN-induced neurotoxicity are mediated by nitrodative stress induced neuroinflammation and the neuroprotective role of sesamol and quercetin should be explored further as effective agents in the management of Huntington's disease.

Disclosures: A. Kuhad: None. **S. Singla:** None. **V. Arora:** None. **K. Chopra:** None.

Poster

538. Neurotoxicity and Neurodegeneration II

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.15/Z18

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: MRC grant

Title: The role of PGC1α and mitochondrial dynamics in lead-induced neurotoxicity

Authors: *A. DABROWSKA, N. HAJJI;
Hammersmith Hosp., Imperial Col. London, London, United Kingdom

Abstract: Mitochondrial dysfunction is a known contributor to many neurodegenerative conditions including Parkinson's disease (PD). Mitochondrial biogenesis, dynamics and function have all been implicated in PD. Chronic exposure to lead (Pb^{2+}) has been shown to correlate with an increased risk of PD. A mechanism mediating this effect remains unknown but existing data suggest that mitochondrial impairment and alterations of cellular calcium balance are involved. In this study I evaluated the effect of Pb^{2+} on mitochondrial biogenesis, function and dynamics in a relevant cellular model, focusing on the role of PGC1α, a transcription coactivator, a known master regulator of mitochondrial biogenesis, also implicated in PD.
N27 dopaminergic neuronal cells were exposed to a range of lead (II) acetate concentrations (5-100 μM) for 48 hours. Mitochondrial membrane potential (ΔΨm) loss and cell death were measured by flow cytometry analysis. At low Pb^{2+} concentrations (<100μM) we observed ΔΨm loss but no cell death and a dose-dependent increase in PGC1α expression. At high Pb^{2+} concentrations (≥100μM) a reduction of ΔΨm and increased cell death were observed, accompanied by a reduction of PGC1α expression. Knockdown of PGC1α rendered the cells more susceptible to lead-induced loss of ΔΨm and led to fragmentation of mitochondria, as indicated by immunostaining. Independent knockdown of each component the Fis1/Bap31 complex has a neuroprotective effect against Pb^{2+}. Given that Fis1 is a mitochondrial fission protein and Bap31 is ER-localised and they bridge the mito-ER platform for apoptosis initiation, Pb^{2+} induced neurotoxicity could be due to disruption of mitochondrial dynamics.

Disclosures: A. Dabrowska: None. **N. Hajji:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.16/AA1

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: Grants R01 AG021975(S. A. F.)

U0128583(S.A.F.),P30-AG028748(UCLA Older Ameri-cansIndependenceCenter;NIA)

RC1 AG035878(S. A. F.and G. M. C.)

AT006816(G. M. C.),P01 AG16570(UCLA Alzheimer Disease Research Center; to G. M. C.)

Veterans Affairs Merit grant (G. M. C). Veterans Affairs Merit grant X001257 (S. A. F.).

UCLA Mary S. Easton Translation Center (S. A. F., G. M. C.)

China Scholarship Council Scholarship

Title: Novel mechanism of curcumin anti-inflammatory activity and control upstream of nf-kappa b activation in brain aging and neurodegenerative disease

Authors: *Q. CHEN[1,2], P. MAITI[1], X. ZUO[1], F. YANG[1], Q. MA[1], Z. YU[2], G. M. COLE[1], S. A. FRAUTSCHY[1];
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Abstract: Neuroinflammation is implicated in the pathogenesis of many neurodegenerative diseases of aging. Curcumin has an outstanding safety profile and pleiotropic beneficial activities in animal models of neurodegenerative and other CNS syndromes and inflammatory diseases. A large subset of beneficial effects derive from curcumin's anti-inflammatory properties attributed to inhibition of NF-κB regulated glial activation. Further, CNS NF-κB activation appears to play an important role in regulating hypothalamic neuroendocrine changes promoting systemic aging and reducing longevity. Our previous data in multiple AD models and aged animals shows that levels of iNOS,COX2, IL-1β and TNF –α, all pro-inflammatory mediators induced by NF-κB, were suppressed in vivo by curcumin treatment with brain IC50's in the 1-2 μM range. As NF-κB is not a direct target of curcumin, the direct targets underlying NF-κB inhibition by curcumin

remain unclear. The IKK complex that sequesters NF-κB in the cytosol contains and requires CDC37/Hsp90 for its assembly so that Hsp90 inhibitors can prevent activation. Here, using GST-pull down assays, we prove that curcumin at CNS concentrations achieved in vivo directly suppresses CDC37/ Hsp90 complex formation. Complex suppression is partial and occurs below concentrations required to inhibit Hsp90 ATPase activity. We also use co-IP to show that this partial inhibition of complex formation occurs in vivo in wild type and transgenic model animals treated with curcumin. Because the CDC37/Hsp90 complex also regulates many client kinases involved in cancer and other age-related diseases, these results may help explain the remarkable pleiotropic protective activities of curcumin. The concentrations required for human CDC37/Hsp90 complex inhibition can be produced in curcumin treated people. Therefore, our data provides a novel mechanism to help explain partial NF-κB inhibition and multiple protective activities of curcumin in vivo.

Disclosures: Q. Chen: None. **P. Maiti:** None. **X. Zuo:** None. **F. Yang:** None. **Q. Ma:** None. **Z. Yu:** None. **G.M. Cole:** None. **S.A. Frautschy:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.17/AA2

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AG030399

NIH Grant AG037814

Title: IL-17A and IL-17RA in mouse brain: Their expression and pathophysiological analyses by AAV-mediated overexpression of IL-17A

Authors: *J. YANG[1], J. KOU[1], R. LALONDE[2], K.-I. FUKUCHI[1];
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Abstract: Interleukin-17 (IL-17) is a proinflammatory cytokine mainly expressed by T helper 17 (Th17) cells. Recent studies, however, show that IL-17A is expressed by several different immune cell types and that its receptor (IL-17R) is expressed in many cell types including epithelial cells, endothelial cells, fibroblasts and myeloid cells. Particularly, IL-17 is increasingly drawing attention as a crucial factor in inflammatory disorders of the nervous system. Additionally, the signaling pathways of IL-17 are being considered as therapeutic targets of such

diseases including multiple sclerosis, stroke, and brain tumors. Therefore, we have studied expression of IL-17A and IL-17RA in the brains from C57BL/6 mice at 1 and 10 months of age. Expression of IL-17A was studied by in situ hybridization with RNA oligonucleotide probes and IL-17RA expression was determined by both immunohistochemistry and western blot analysis. IL-17A expression was found in the motor cortex, priform cortex, CA1 field and granular layer of the hippocampus at 1 and 10 months of age. IL-17RA was barely expressed in the brain at 1 month and strongly expressed in the CA2 and CA3 fields of the hippocampus and the thalamus at 10 months. To further investigate the functions of IL-17A, we produced recombinant adeno-associated virus serotype 5 (rAAV5) encoding mouse IL-17A and injected the virus into the right lateral ventricle of the mouse brain. Three months after injection, high expression of IL-17A was found throughout the hippocampus and partly in the neocortex in both brain hemispheres. We are currently studying the effects of IL-17A overexpression on physiological functions, gene expression and inflammation in these experimental animals.

Disclosures: J. Yang: None. **J. Kou:** None. **R. Lalonde:** None. **K. Fukuchi:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.18/AA3

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R21NR012845

Title: Developing an animal model of fatigue: Neuroinflammation by pro-inflammatory cytokines

Authors: *D. BONSALL, G. B. MCKAY-CORKUM, P. C. MOLYNEUX, M. E. HARRINGTON;
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Abstract: Fatigue is a debilitating symptom prevalent in many disease states including Multiple Sclerosis, Parkinson's disease and Chronic Fatigue Syndrome. The cause of fatigue remains unclear, however, neuroinflammation has been strongly implicated in its development. Here we present our current progress in building an animal model of fatigue that is instigated through the actions of the pro-inflammatory cytokine Interleukin 1-beta (IL-1β). Peripheral administration of IL-1β is able to significantly reduce running wheel activity in middle-aged (6-12 months old) C57Bl/6 female mice while general locomotor activity remains unaltered. Furthermore, we do not observe weight loss or fever associated with this treatment, as determined through the use of

abdominal telemetry probes. We demonstrate dose-, gender- and age-dependent differences in the response to IL-1β given peripherally. In addition, intracerebroventricular administration of IL-1β supports a centrally mediated mechanism which is explored further using immunohistochemistry. We assess IL-1β-related changes in microglial activation and in the expression of pro-inflammatory cytokines throughout the brain, in regions linked to the arousal system and motivational pathways.

Disclosures: D. Bonsall: None. **G.B. McKay-Corkum:** None. **P.C. Molyneux:** None. **M.E. Harrington:** F. Consulting Fees (e.g., advisory boards); Merz Pharmaceuticals GmbH.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.19/AA4

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: DK42932

HL53740

MH079079

CA21765

Title: Loss of CA3 pyramidal neurons in mice deficient for SCYL2, a clathrin coated vesicle-associated protein pseudokinase

Authors: *S. PELLETIER[1], S. GINGRAS[1], S. HOWELL[2], L. EARLS[3], R. SMEYNE[3], S. ZAKHARENKO[3], J. N. IHLE[2];
[1]Immunol., [2]Biochem., [3]Developmental Neurobio., St. Jude Children's Res. Hosp., Memphis, TN

Abstract: SCYL2/CVAK104 is a clathrin coated vesicle (CCV)-associated protein pseudokinase of unknown function. Using biochemical, cell biological and genetic approaches, we show here that SCYL2 is an evolutionarily conserved protein that interacts specifically with the clathrin/AP2 complex and plays a critical role for the normal development and function of the nervous system and for suppressing excitotoxicity during synaptogenesis. Ubiquitous and neural-specific deletion of *Scyl2* in mice caused severe neurological disorders that resulted in the death of a majority of newborn mice. In surviving animals, SCYL2 loss caused growth retardation and symptoms of neurological disorders that were associated with the loss of various neuronal populations, most notably the CA3 pyramidal neurons of the hippocampus. Loss of CA3 neurons

occurred rapidly during the early postnatal development and was the result of an apoptotic process that was reversed by *Bax* deficiency or glutamate receptor antagonism. Ubiquitin-neurotransmitter receptor-positive aggregates and abnormal neurotrophin receptor localization in the CA3 region of the hippocampus were conspicuous. Strikingly the phenotypic changes observed in *Scyl2*-deficient mice is similar to those found in mice deficient for components of the Endosomal Sorting Complexe Required for Transport (ESCRT)-0, a protein complex required for endosomal sorting of endocytosed receptor, suggesting that SCYL2 may act along this pathway. Consistent with this, Affinity purification, biochemical fractionation and microscopic studies revealed that SCYL2 is a clathrin/AP2-associated protein that localizes pre- and post-synaptically and facilitates clathrin-mediated endocytosis and degradation of membrane receptors. Given the role of neurotrophin and neurotransmitter receptors in regulating neuronal cell death and survival, loss of CA3 neurons in *Scyl2*-deficient mice may result from their inadequate regulation.

Disclosures: S. Pelletier: None. **S. Gingras:** None. **S. Howell:** None. **L. Earls:** None. **S. Zakharenko:** None. **J.N. Ihle:** None. **R. Smeyne:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.20/AA5

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01-HL060922

NIH R01 HL104173

Title: Astrocyte activation in white matter injury associated with cardiopulmonary bypass in a mouse brain slice model

Authors: K. AGEMATSU[1], L. KOROTCOVA[2], V. GALLO[2], N. ISHIBASHI[2], *R. A. JONAS[2];
[1]Children's Natl. Med. Ctr., Washington, DC; [2]Children's Natl. Med. Ctr., WASHINGTON, DC

Abstract: White matter (WM)
injury is common after cardiac surgery in neonates and young infants with severe/complex congenital heart disease (CHD). The cellular mechanisms that govern the ***response*** of the brain to surgery-induced WM injury remain largely unexplored. We studied WM astrocytes in a rodent brain slice model of brain injury caused by cardiopulmonary bypass (CPB). The

GFAP-GFP mouse was used to identify WM astrocytes. Living brain slices of postnatal day 11 mice, developmentally equivalent to the human newborn, were transferred to a closed chamber perfused with artificial cerebrospinal fluid under controlled temperature/oxygenation to reproduce conditions of CPB. After cooling to 15, 25, and 35^{o}C, 60 min oxygen-glucose deprivation (OGD) was performed to simulate circulatory arrest. An increase in WM caspase3^{+} cell number at 20hrs after reperfusion was identified in all OGD groups, including 15^{o}C ($p<.001$), but not in Control.
The number was positively correlated with temperature during OGD (rho=.66, $P<.01$), demonstrating that temperature-dependent WM injury is similar to that seen in large animal models of CPB. Caspase3^{+} astrocytes significantly increased at 20hrs after 25 and 35^{o}C OGD ($p<.001$), but not in 15^{o}C OGD, indicating that deep hypothermic OGD did not induce astrocyte injury. When the relation between astrocytes and WM apoptosis was investigated under hypothermia at 15^{o}C and 25^{o}C, GFAP^{+} astrocyte number was positively correlated with caspase3^{+} cell number at 20hrs after reperfusion ($r=.77$, $p<.001$). This finding demonstrates that astrocytes play an important role in WM injury associated with CPB using hypothermia. Therefore, future studies will target the molecular and cellular interaction between WM astrocytes and damaged cells with the goal of developing a novel pharmacological approach for reducing the risk of WM injury during cardiac surgery.

Disclosures: K. Agematsu: None. **R.A. Jonas:** None. **L. Korotcova:** None. **V. Gallo:** None. **N. Ishibashi:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.21/AA6

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Single cell imaging identifies toxic effects on functions relevant for neuronal communication

Authors: J. SISNAISKE, V. HAUSHERR, C. VAN THRIEL, *N. SCHOEBEL;
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Abstract: Neurons form delicate networks of interconnected cells whose communication requires intact neurites and a finely-tuned interplay of neurochemical processes at the synapse.

Because of their highly specialized morphological and functional features, neurons are especially vulnerable to the harmful effects of environmental and occupational toxins. Neurotoxicology is challenged with the need for sensitive animal-free in vitro assays to identify and estimate the neurotoxic potency of a plethora of chemicals. Typically, cell viability assays are used to evaluate neurotoxic effects in vitro. However, it has been shown that neurite degeneration may occur at concentrations that do not or only weakly affect the viability of neuronal cells. Thus, the outcome of cell viability assays may underestimate the neurotoxic potency of a given substance. Following the notion that some toxins specifically affect neurites, we evaluated whether processes critical for inter-neuronal communication might also be more sensitive to toxin treatment than cell viability. Here, we assessed the functionality of neurochemical processes by fluorescence-based optical imaging (calcium imaging) of primary cortical neurons of mice in vitro. After pretreatment with substances of different neurotoxic potential, functional responses to depolarization and glutamate stimulation were assessed. For the direct comparison of assay sensitivities, neurons were subjected to a cell viability assay (cell titer blue, CTB) in parallel. We compared the dose-response functions for toxic effects measured by both assays. For a first group of substances encompassing known non-neurotoxins (e.g. mannitol), dose-response functions and EC50 values of the functional imaging data paralleled that of the CTB. For a second group of substances including known neurotoxins (e.g. MPP+), dose-response functions obtained by functional imaging were shifted to the left by one to several orders of magnitude in comparison to the CTB. These results clearly indicate a higher sensitivity of neuronal functional parameters to certain neurotoxins than cannot be achieved by CTB. Furthermore, we suggest that the proper estimation of in vitro neurotoxic effects requires multiple test assays, with calcium imaging being a useful tool for the identification of neurotoxic effects on parameters relevant for neuronal communication.

Disclosures: J. Sisnaiske: None. **V. Hausherr:** None. **C. van Thriel:** None. **N. Schoebel:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.22/AA7

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sigma-1 Receptor Chaperone plays an essential role in neuronal function by regulating p35/CDK5

Authors: *S.-Y. A. TSAI[1], N. R. KLAUER[2], T.-P. SU[1];
[1]Cell. Pathobiology Sec Integrative Neurosci. Br,, NIDA-IRP, NIH, BALTIMORE, MD; [2]Univ. of Minnesota Med. Sch., Minneapolis, MN

Abstract: The endoplasmic reticular protein sigma-1 receptor (Sig-1R) is a novel molecular chaperone and has been implicated in central nervous system diseases such as Alzheimer's disease, depression, and drug abuse. Imaging studies have demonstrated patients with either Alzheimer's or Parkinson's disease exhibit reduced Sig-1R protein levels. Sig-1Rs are particularly enriched in the mitochondria-associate ER membrane (MAM); however, little is known about Sig-1R's specific role in neurodegeneration. This study examined the effects of Sig-1R on the regulation of the cyclin-dependent kinase (CDK)5 activator, p35. CDK5 hyperactivity has long been associated with the etiology of neurodegenerative disease due to its role in tau phosphorylation and subsequent aggregation. Neuronal damage results in release of Ca^{2+} into the cytoplasm activating the calpain protease which cleaves p35 into a more potent CDK5 activator, p25. Due to the detrimental effects of CDK5 hyperactivity, the expression of p35 is tightly regulated and exhibits a short half-life of approximately 20 minutes. Therefore, we explored specifically the role of Sig-1R in p35 degradation. Our initial results indicated that Sig-1R KO mice exhibited elevated p35 and phosphorylated tau expression. We found that this alteration in basal protein p35 expression is likely due to a reduced basal degradation rate of p35 in siSig-1R treated cells. Furthermore, upon calcium induced calpain activation, Sig-1R KO brain homogenates also displayed elevated p25 accumulation. siSig-1R neurons also exhibited increased CDK5 activity following glutamate insult. Together, these results suggest that Sig-1R is involved in the degradation of p35, which may explain the increased susceptibility of Sig-1R deficient cells to neurotoxic insults.

Disclosures: S.A. Tsai: None. **N.R. Klauer:** None. **T. Su:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.23/AA8

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: Aim for the Top University Plan, Ministry of Education, Taiwan

Title: Effects of soluble epoxide hydrolase inhibitor on NMDA-induced excitotoxicity and BDNF expression in cortical neurons

Authors: *Y.-M. KUO[1,2], T.-S. LEE[1,3], Y.-H. LEE[1,3];
[1]Dept. and Inst. of Physiology, Natl. Yang-Ming Univ., Taipei City, Taiwan; [2]Dept. of Anesthesiol., Taipei Veterans Gen. Hosp. and Natl. Yang-Ming Univ. Sch. of Med., Taipei, Taiwan; [3]Brain Res. Center, Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Soluble epoxide hydroxylase (sEH) is a dual activity enzyme with the C-terminal hydrolase activity mediating metabolic degradation of arachidonic acid into epoxyeicosatrienoic acids (EETs). Pharmacologic inhibition and genetic deletion of sEH have been shown to reduce infarct size after experimental stroke. The neuroprotective effect of sEH inhibitor was attributed to its anti-inflammatory effect, but how its direct effect on neuronal sEH contributes to the neuronal survival remains unclear. The present study investigated the effect of sEH inhibition on excitotoxicity-induced neuronal death and neurotrophic factor expression in primary cortical neurons. We used 9-10 days in vitro primary cortical neuron cultures derived from E17 rat brains. The C-terminal sEH inhibitor 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA, 10μM) was used to pretreat the culture prior to the 24h NMDA treatment. Neuronal death was determined by lactic dehydrogenase (LDH) release assay, and viability was determined by WST-1 assay. Brain-derived neurotrophic factor (BDNF) mRNA expression was measured by quantitative RT-PCR. The results show that 10μM AUDA pretreatment significantly enhanced 20 and 50μM NMDA-induced LDH release, which was in accordance with results obtained in the viability assay by WST-1 assay. Real-time PCR analysis revealed that AUDA also enhances 20 and 50μM NMDA-induced BDNF mRNA expression. Thus, inhibition of the neuronal sEH hydrolase activity by AUDA seems to enhance NMDA receptor mediated excitotoxicity and BDNF expression. Of note, inhibition of sEH might counteract its anti-inflammation-dependent neuroprotection when applied to brain injuries involving excitotoxicity-induced neurodegeneration.

Disclosures: Y. Kuo: None. **T. Lee:** None. **Y. Lee:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.24/AA9

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: CSIR Project BSC0123

CSIR Research fellowship

Title: Perturbation in the miRNA gene regulation associated with neurodegeneration and neurogenesis

Authors: *A. CHOUDHARY, R. ROSHAN, K. SONI, A. R. SINGH, S. SHRIDHAR, R. DEY, S. SIVASUBBU, B. PILLAI;
Inst. of Genomics and Integrative Biol., New Delhi, India

Abstract: Spinocerebellar ataxia 17(SCA17) is a type of polyglutamine disease characterized by CAG repeat expansions near the N-terminus of the general transcription factor, TATA Binding Protein (TBP). Expansion of the polyglutamine repeat in TBP increases its propensity to form aggregates in neuronal cells and leads to the gradual death of neurons causing ataxia, dementia and involuntary movements in the patients. To understand the molecular mechanisms involved in the pathogenesis of SCA17, we have created and validated a cellular model by expressing human TBP with normal and expanded glutamine repeats in Neuro2A cells. Using gene expression analysis we have earlier shown several differentially expressed miRNAs in neuronal cells undergoing apoptosis due to the expression of the mutant TBP. We predicted the putative targets of these miRNAs and experimentally validated a set of calcium signalling genes as target genes including Inositol triphosphate receptor1 (ITPR1), Syntaxin1a (STX1A) and AKT3. These genes contribute in neuronal calcium signalling and neurotransmitter release and hence are important in the context of neurodegeneration. Also, in the mRNA expression profiles we found a set of differentially expressed mRNAs which are involved in the IFNϒ signalling pathway. We also observed that IFNϒ can regulate the expression of miRNAs in both STAT1 dependent or STAT1 independent manner and thereby regulate the cellular targets of miRNAs. Integrating mRNA and miRNA gene expression profiles, we are able to demonstrate a novel mRNA- miRNA interaction network that plays important role in cellular model of neurodegeneration.
We also emplyoed a zebrafish based model to study neurogenesis. We have studied the effect of several miRNAs on the zebrafish brain development. Interestingly,knockdown of miR-34a ,a cell cycle regulator and modulator of notch signalling pathway resulted in the defects in the early development of hindbrain of zebrafish. Phenotypes of knockdown of other miRNAs during zebrafish development are also discussed.

Disclosures: A. Choudhary: A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology. **R. Roshan:** A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology. **K. Soni:** A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology. **A.R. Singh:** A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology. **S. Shridhar:** A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology. **R. Dey:** A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology. **S. Sivasubbu:** A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology. **B. Pillai:** A. Employment/Salary (full or part-time):; CSIR-Institute of Genomics and Integrative Biology.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.25/AA10

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIEHS R00ES015428

Title: Dopamine transporter (DAT) expression in the medial prefrontal cortex and striatum of Long-Evans rats is affected by perinatal exposure to polychlorinated biphenyls

Authors: M. M. MILLER, J. L. NELMS, A. E. MEYER, *H. J. SABLE;
Dept. of Psychology, Univ. of Memphis, Memphis, TN

Abstract: Due to their persistence and ubiquity in the environment as well as their current presence as an unintentional byproduct of manufacturing, polychlorinated biphenyls (PCBs) assert a significant human health threat. Modifications in brain dopamine (DA) concentrations following perinatal exposure to PCBs in rats have been shown to persevere into adulthood, and we have demonstrated perinatal PCB exposure alters the behavioral response to psychostimulant drugs known to act on the DA system during adulthood. Previous findings demonstrate that adult exposure to PCBs inhibits DAT expression, but the effects on DAT following perinatal exposure are less understood. The purpose of this project was to determine the effects of prenatal and early postnatal exposure to an environmentally relevant mixture of PCBs on DAT expression in the medial prefrontal cortex (mPFC) and striatum in weanling rats. Long-Evans dams were orally exposed to 0, 3 or 6 mg/kg/day of an environmentally relevant mixture of PCBs beginning at 4 weeks prior to breeding and continuing until litters were weaned on postnatal day 21. At weaning, the mPFC and striatum were extracted from one male and one female per litter for Western blot analysis. It was hypothesized that DAT expression in animals exposed to PCBs during the perinatal period would be reduced relative to non-PCB-exposed control rats. Results confirmed the hypothesis and demonstrated that weanlings exposed to 6 mg/kg/day PCBs had less DAT expression compared to rats in the 3 mg/kg/day group and non-PCB-exposed control group in both mPFC and striatal samples. These findings indicate that perinatal exposure to an environmentally relevant PCB mixture results in an outcome similar to what has been reported in adult animals exposed to PCBs, and suggest a possible mechanism for the differential response to psychostimulant drugs observed in rats perinatally exposed to PCBs.

Disclosures: M.M. Miller: None. **J.L. Nelms:** None. **A.E. Meyer:** None. **H.J. Sable:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.26/AA11

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR Grant MOP-14828

Title: Differential functions of infiltrating macrophages and resident microglia after spinal cord injury

Authors: *A. D. GREENHALGH, S. DAVID;
McGill Univ., Montreal, QC, Canada

Abstract: Macrophages in the injured spinal cord arise from resident microglia and infiltrating, peripherally derived monocytes. It is still not clear if macrophages derived from these two populations differ in their roles after spinal cord injury (SCI). The aim of this study was to investigate the contribution to the phagocytic response and the clearance of damaged axons by macrophages derived from resident microglia in comparison to macrophages of a peripheral, blood-borne origin. The LysM-eGFPki transgenic mouse tags haematogenous macrophages, but not microglia, and allows the study of these two previously indistinguishable cell populations without the need for chimeric experiments. We used a combination of immunofluorescence, flow cytometric and neuronal tracing techniques (using fluorescently labelled dextran to trace axons in the dorsal column) we show that microglia contact damaged axons early (24 h) after SCI and are the predominant type of macrophage to contain phagocytic material at 3 days. Thereafter, infiltrating macrophages become the predominant cell in contact with degenerating axons and contain more phagocytic material. Furthermore, after phagocytosing myelin in vitro, bone marrow derived macrophages are much more susceptible to apoptotic and necrotic cell death than CNS microglia. These data show that peripherally derived macrophages are the main cell type to phagocytose degenerating axons after CNS injury despite the presence of microglia, but may not be as well equipped to process the phagocytosed material. Overall, these data highlight the differential roles played by different types of macrophages, depending on their origin, and provide further information for cell specific targeting of inflammatory response after SCI.

Disclosures: A.D. Greenhalgh: None. **S. David:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.27/AA12

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: The role of microRNAs (miRNAs) in the inflammatory processes related to ageing: A pilot study

Authors: *A. M. FLOREA;
Dept. of Envrn. Toxicology, Univ. of Trier, Trier, Germany

Abstract: Chronic inflammation is an important phenomenon that takes place in the ageing processes of the brain. MiRNAs are gene regulators that play a role in post-transcriptional repression of many genes. A new body of evidence shows that miRNA are able to property finely-tune gene expression of genes that are important for immune system regulation (Olivieri et al., 2013) thus, the precise control for proper activity if immune response could be affected. For instance the expression of hsa-miR-21 was associated with ageing in humans which in turn is a validated target of the TGF-βR2 mRNA that is as well deregulated in inflammatory processes related to ageing. Thus, miR-21 may represent a new biomarker of inflammation (Olivieri et al., 2012; 2013). Also in vitro cellular senescence has been associated with inflammatory response and miRNA deregulation (e.g. miR-21, miR-214, miR-92; Rippe et al., 2012). In this study we investigate the involvement of miRNAs in ageing related inflammatory processes with a focus on the brain. Literature and data mining are used together with own investigation in order to underline new prove of evidence supporting the hypothesis. Olivieri et al., 2013; doi: 10.1186/1742-4933-10-11.Olivieri et al., 2012; doi: 10.1016/j.mad.2012.09.004. Rippe et al., 2011; doi: 10.1016/j.exger.2011.10.004.

Disclosures: A.M. Florea: None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.28/AA13

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: Korea Healthcare technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A120202)

Title: Effects of cytidine 5′-diphosphocholine (CDP-choline) on hypoglycemia-induced neuron death

Authors: *J. KIM[1], B. CHOI[1], H. KIM[1], J. YOO[1], M. SOHN[2], H. CHOI[1], H. SONG[1], S. SUH[1]; [1]Dept. of Physiol., Hallym University, Col. of Med., Chuncheon, Korea, Republic of; [2]Inha Univ., Incheon, Korea, Republic of

Abstract: Diabetic patients who attempt strict management of blood glucose levels frequently experience hypoglycemia. Severe and prolonged hypoglycemia causes neuronal death and cognitive impairment. There is no effective tool for prevention of these unwanted clinical sequelae. Citicoline (CDP-choline; cytidine 5'-diphosphocholine) is an important intermediate in the biosynthesis of cell membranes phospholipids. Citicoline serves as a choline donor in the metabolic pathways for biosynthesis of acetylcholine and neuronal membrane phospholipids, mainly phosphatidylcholine. The ability of citicoline to reverse the neuronal injury has been tested in animal models of cerebral ischemia and also has been performed clinical trial in stroke patients. However, no previous report has examined the effect of citicoline on hypoglycemia-induced neuron death. To clarify the therapeutic potency of citicoline on hypoglycemia-induced neuron death, we used an animal model of insulin-induced hypoglycemia. Acute hypoglycemia was induced by intraperitoneal injection of human insulin (10 U/kg), and then iso-electricity was maintained for 30 minutes. Citicoline injection was started immediately after hypoglycemia (500mg/kg, i.p.). ROS production was evaluated at 3 hours after hypoglycemia. Neuronal injury, microglia activation and BBB disruption were evaluated at 1 week after hypoglycemia. Here we found that post-treatment of citicoline showed significant less ROS production, neuron death, microglia activation and BBB disruption in the hippocampus compared to vehicle treated group. Taken together, these results suggest that neuronal membrane stabilization by citicoline can rescue neurons after severe hypoglycemia as seen in several ischemia studies. The present study suggests that citicoline may have a high therapeutic potential to reduce hypoglycemia -induced neuronal death.

Disclosures: J. Kim: None. **B. Choi:** None. **H. Kim:** None. **J. Yoo:** None. **M. Sohn:** None. **H. Choi:** None. **H. Song:** None. **S. Suh:** None.

Poster

538. **Neurotoxicity and Neurodegeneration II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 538.29/AA14

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: RO1 AG030331

RO1 AG037320

Title: Neuroinflammation: pathological mechanisms and the beneficial role of insulin therapy

Authors: *L. ADZOVIC[1], S. C. HOPP[2], R. M. KAERCHER[1], S. E. ROYER[2], H. M. D'ANGELO[2], G. L. WENK[1];
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Abstract: Chronic neuroinflammation is associated with many neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. It produces an elevation of pro-inflammatory cytokines, an impairment of spatial memory or motor function and a dysregulation of diverse neurotransmission in selectively vulnerable brain regions. The role of insulin receptor (IR) in the brain is still not clear and several studies suggested it may have a neuroprotective function. Our work analyzed if insulin has a role in neuroprotection by preventing the neuroinflammatory response to lipopolysaccharide (LPS). In this study, we investigated the time course of behavioral, biochemical and pathological changes relative to different brain regions including the hippocampus, substantia nigra and locus coeruleus following either experimentally induced chronic neuroinflammation or neuroinflammation associated with normal aging. Neuroinflammation was created by infusing LPS continuously for four weeks into the 4th ventricle of young (3 months) rats. The neuroprotective actions of insulin were investigated. All rats underwent behavioral testing (Morris water maze, MWM and forced swim, FS). Afterwards, the hippocampus and brainstem were examined by immunohistochemistry the evidence for cell loss (NeuN, GFAP and Caspase-3) and by qRT-PCR to determine gene expression of TNF-α, IL-1ß, IL-10, TGF-ß, TLR4, PPARy, BDNF and GLT1. The results showed that young and middle-aged rats exposed to LPS performed poorly in the MWM as compared to the controls; all aged rats were impaired in MWM task. The gene expression of pro- and anti-inflammatory markers varied depending on the presence of LPS or insulin and the age of the rat.

Disclosures: L. Adzovic: None. **S.C. Hopp:** None. **R.M. Kaercher:** None. **S.E. Royer:** None. **H.M. D'Angelo:** None. **G.L. Wenk:** None.**Poster**

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.01/AA15

Topic: C.14. Sensory Disorders

Title: Baseline PAG cerebral blood flow predicts pain phenotype in a healthy human model of central sensitisation

Authors: *M. MEZUE, V. WANIGASEKERA, M. KELLY, M. CHAPPELL, I. TRACEY; Nuffield Dept. of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom

Abstract: The periaqueductal grey (PAG) has been implicated in descending pain modulatory processes in humans and is thought to be involved in bi-directional pain control. Previous studies have shown the importance of the PAG and other brainstem structures in the maintenance of central sensitisation via descending connections with dorsal root ganglion cells in the spinal cord. Here, we use a novel arterial spin labelling (ASL) fMRI approach and a model of central sensitisation to investigate the importance of inter-individual differences in PAG perfusion at baseline, and its relationship to pain vulnerability in a healthy population.

Method

20 healthy subjects were scanned in two separate sessions in a 3T Verio MRI scanner. In the first session (BL), a 10 minute baseline perfusion fMRI scan (pseudo-continuous ASL, 5 inversion times) was taken, with subjects in a non-sensitised state. In the second session (CS), subjects were scanned 90 minutes after 1% capsaicin cream had been applied on the right leg.

Image analysis was performed with FSL tools. Absolute cerebral blood flow (CBF) was calculated by using a Bayesian inference tool (BASIL) to iteratively calculate blood magnetization kinetics across all voxels for each TI. CBF values were extracted from PAG voxels (for each subject's BL session).

Ongoing and evoked pain scores were evaluated using visual analogue. In the sensitised state, a hyperalgesia score was defined by taking the difference between subject ratings of perceived unpleasantness to elicited punctate stimuli (512 mN probe) before and after capsaicin application.

Results

In the CS session, subjects reported (mean[/100] ±SD) ongoing pain of 18.3 ±11.4 and developed punctate unpleasantness hyperalgesia of 28.3 ±18.1.

Baseline PAG perfusion correlated strongly with ongoing pain scores (average of pre- and post-scan) ($r(18)=0.53$, $p<0.05$) and with punctate hyperalgesia scores ($r(18)=0.53$, $p<0.01$).

We also show that inter-individual baseline PAG CBF values correlate with decreases in perfusion in the CS state (compared to BL) in the contralateral hypothalamus, hippocampus, amygdala and bilateral insula (whole-brain cluster corrected: $Z>2.3$, $p<0.05$).

Discussion

We show that CBF in a key nucleus of the descending pain system predicts individual behavioural and neural responses in the central sensitised state. This positive correlation may indicate that individuals with more facilitatory drive from the PAG at baseline are more vulnerable to pain in an injured state. Furthermore, these individuals may also exhibit increased brainstem plasticity leading to decreased PAG inhibition and hypoperfusion of connected subcortical areas in the CS state.

Disclosures: M. Mezue: None. **V. Wanigasekera:** None. **M. Kelly:** None. **M. Chappell:** None. **I. Tracey:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.02/AA16

Topic: C.14. Sensory Disorders

Support: Supported by the German research council DFG NA 970 1/1

Title: Differential changes of axonal excitability of afferent C-fibers associated with different mutations of NaV1.7 in erythromelalgia patients

Authors: *B. NAMER[1], K. ØRSTAVIK[2], R. SCHMIDT[5], I. KLEGGETVEIT[2], C. WEIDNER[1], C. MØRK[3], K. KVARNEBO[4], Z. ZHANG[6], H. SALTER[6], T. H. CARR[7], S. G. WAXMAN[8], H. O. HANDWERKER[1], E. TOREBJÖRK[5], E. JØRUM[2], M. SCHMELZ[9];
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Abstract: Patients with distal limb pain carrying a diagnosis of erythromelalgia (EM) were investigated by microneurography to record action potentials from unmyelinated nerve fibers (C-units) in cutaneous fascicles of the peroneal nerve. Three patients had mutations in sodium channel (NaV) 1.7 (I848T, I228M, P187L) while no mutations of coding regions of NaVs were found in 4 EM patients.
Recorded C-fibers were classified according to responsiveness to von Frey stimulation (75 mN) and activity-dependent conduction velocity slowing (ADS). ADS and excitability changes following single action potentials (recovery cycles) were tested in single fibers with electrical stimulation in their cutaneous receptive field.
In the patient with mutation I848T all mechano-sensitive C-nociceptors (CM) were characterized by activity-dependent speeding of conduction rather than ADS. In contrast, sympathetic fibers showed the expected ADS. In the recovery cycles, the mechano-sensitive C-fibers had an early subnormality that changed into supranormal conduction around 700 ms after the preceding action potential.
In contrast, no differences in ADS or recovery cycles were found in CM nociceptors between EM patients without mutations, with mutations I228M and P187L, and healthy controls. Irrespective of NaV1.7 mutations, in the EM patients about 30-50% of the fibers showed

spontaneous activity which could be evoked by heating the skin of the receptive field in some fibers. In these patients, only the atypical afferent C-fibers with spontaneous activity and/or mechanical sensitization showed signs for a more moderate depolarization in the recovery cycles.
Our data suggest a massive axonal depolarisation in the patient with I848T mutation which corresponds to patch clamp data and the severe clinical symptoms of the patient. In the patients with other NaV1.7 mutations, no such specific changes could be observed. However, there were signs of moderate depolarisation in hyperexcitable nociceptors in the patients with and without NaV1.7 mutations (I228M, P187L).
Using microneurography, we find a generic pattern of hyperexcitability in EM patients and changes suggestive of axonal depolarization irrespective of NaV1.7 mutations. Mutation specific changes were restricted to the clinically most severe case of EM with I848T mutation.

Disclosures: B. Namer: None. **C. Weidner:** None. **H.O. Handwerker:** None. **K. Ørstavik:** None. **E. Jørum:** None. **I. Kleggetveit:** None. **R. Schmidt:** None. **E. Torebjörk:** None. **M. Schmelz:** None. **S.G. Waxman:** None. **Z. Zhang:** None. **H. Salter:** None. **T.H. Carr:** None. **C. Mørk:** None. **K. Kvarnebo:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.03/AA17

Topic: C.14. Sensory Disorders

Support: Swedish Governmental Agency for Innovation Systems and the Swedish Research Council grant number P29797-1

Lars Hiertas Minne foundation F02009-0695

Signe och Olof Wallenius foundation R103

Title: Proteins increased in cerebrospinal fluid after spinal cord stimulation elucidate human neuropathic pain relief mechanisms

Authors: LIND[1,2], M. SJÖDIN[1], L. KATILA[2], M. WETTERHALL[1], T. GORDH[2];
[1]Dept. of Analytical Chem., Uppsala, Sweden; [2]Dept. of Surgical Sciences, Uppsala Univ., Uppsala, Sweden

Abstract: Objective: To elucidate the analgesic mechanism of spinal cord stimulation (SCS) in patients with neuropathic pain.
Introduction: SCS is a treatment option available to some patients with otherwise treatment resistant neuropathic pain. More than 50% pain relief is obtained during SCS trial stimulation for 60-70% of the patients. Though successfully used since the 1960s, the analgesic mechanism of SCS in neuropathic pain remains unknown. For responders, SCS is in many respects an ideal treatment. However, it is not known why 30-40% of patients do not respond. Also, SCS is initially costly, and rather work intensive as it includes trial stimulation, surgery, battery changes and in some cases lead migration correction surgery. Understanding of the mechanism of SCS analgesia in neuropathic pain could lead to further improvements of current treatments of neuropathic pain and reveal previously unknown therapeutic targets directly in patients with successful treatment. It is plausible that the cerebrospinal fluid (CSF) mirrors parts of mechanism relevant molecular changes taking place in the CSN during stimulation.
Methods: CSF samples were collected from SCS-responsive neuropathic pain patients (n=12) at two separate occasions, first after the SCS had been turned off for 48 h, and then after the SCS had been used normally for three weeks. The off- and on-state proteomes for each patient were relatively quantified using a mass spectrometry shotgun proteomic approach.
Results: A panel consisting of seven proteins, 5 up-regulated (angiotensinogen, kallikrein-6 , amyloid beta A4 protein A4, ganglioside GM2 activator SAP3 and Ly-6/neurotoxin-like protein 1) and 2 down-regulated (complement C2, insulin-like growth factor-binding protein 6)was found to be significantly altered ($P \leq 0.01$) by pain relieving SCS in humans.
Conclusions: This is to our knowledge the first assumption-free longitudinally designed proteomic study of CSF from neuropathic pain patients using SCS. Our findings are unexpected and open several novel research questions about the therapeutic mechanism of spinal cord stimulation.

Disclosures: Lind: A. Employment/Salary (full or part-time):; Uppsala Berzelii Technology Centre for Neruodiagnostics, Uppsala Unviersity. **M. Sjödin:** None. **L. Katila:** None. **M. Wetterhall:** A. Employment/Salary (full or part-time):; Uppsala Berzelii Technology Centre for Neruodiagnostics. **T. Gordh:** A. Employment/Salary (full or part-time):; Uppsala Berzelii Technology Centre for Neruodiagnostics.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.04/AA18

Topic: C.14. Sensory Disorders

Support: CIHR MOP98006

Title: Gamma oscillations in the somatosensory thalamus of a phantom limb patient

Authors: *D. BASA[1,2], M. HODAIE[3], A. LOZANO[2], W. D. HUTCHISON[2];
[1]Univ. of Toronto, Toronto, ON, Canada; [3]Neurosurg., [2]Toronto Western Hosp., Toronto, ON, Canada

Abstract: Phantom limb is the abnormal perception of a non-existent extremity that occurs in patients who have undergone accidental or clinical amputation of a limb. Thalamocortical dysrhythmia (TCD) has been proposed as a model to explain the intrinsic generation of this percept. TCD refers to the abnormal occurrence of theta-gamma oscillations in deafferented areas of the thalamus. We report the occurrence of gamma oscillations (37Hz) in the somatosensory (ventral caudal - Vc) thalamus of a phantom limb patient whose right arm was amputated at the shoulder. Microelectrode recordings were obtained from 3 patients (phantom limb, post-stroke pain and essential tremor) during surgical targeting procedures for deep brain stimulation implants. A total of 22 ventral caudal (Vc - sensory) and 21 ventral intermediate (Vim - motor) cells were analyzed for oscillations in the gamma band in spikes and local field potentials (LFPs). Spikes were template matched using Spike2 (CED) and spectral analysis of spike trains and LFP signals was performed in MATLAB. In the phantom limb patient, 6 out of 7 sites in left Vc, had ongoing 37Hz gamma oscillatory activity in both spike train and LFPs. Gamma oscillations were strongly coherent between spikes and the LFP at these sites. Post-stroke pain and essential tremor patients showed no significant gamma oscillations in Vc. Sustained gamma oscillations in the phantom limb case were confined to Vc and did not occur in Vim. Moreover, high frequency stimulation (50 uA, 200 Hz, 2 s) at two Vc sites induced the 37 Hz gamma oscillation in both spikes and LFPs. The stimulation was concomitant with the patient's report of paresthesia in the non-existent arm. The results suggest a causal relation between pathological gamma oscillations in the somatosensory thalamus and the conscious perception of the phantom limb.

Disclosures: D. Basa: None. **M. Hodaie:** None. **A. Lozano:** None. **W.D. Hutchison:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.05/BB1

Topic: C.14. Sensory Disorders

Support: Avacen Inc. Research Grant

Title: Significant reduction in fibromyalgia (FM) tender point count, widespread pain index (WPI) and symptom severity (SS) score after one month of treatment with AVACEN thermal exchange system (TES)

Authors: *T. MOELLER-BERTRAM[1], M. G. KINCAID[2], R. BOOHER[3], D. GARCIA[4], L. KY[5], J. M. SCHILLING[3], I. STRIGO[2];
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Abstract: Fibromyalgia (FM) is a common chronic pain disorder that also affects sleep and mood. The diagnosis of FM was classically made using a tender point exam system, but the new American College of Rheumatology Diagnostic Criteria recognize the importance of quantitative measure of widespread pain (WPI) and also incorporates key FM symptoms (SS Score). The treatment of FM remains challenging, and the underlying pathology is poorly understood. Recent evidence implicates involvement of arteriovenous (AV) shunts of the extremities in FM pathology. The AVACEN Thermal Exchange Method (TEM) is a therapeutic medical device and method that manipulates AV shunts in the palm of the hand. TEM noninvasively infuses heat into the circulatory system for whole body treatment. A pilot study using TEM for 10 minutes once a day in 5 FM subjects showed reduction in FM related pain and related symptoms. In a follow up study, 17 subjects (2 male, 15 female) with physician diagnosed FM were enrolled after IRB approval and trained in the use of the TEM device. The modified protocol required use for 15 minutes twice a day for four weeks. Outcome measures included physician assessed FM diagnostic criteria including a tender point exam, WPI and SS score, and weekly self-assessed pain ratings. All assessments were carried out before (n=17) and after (n=14) the treatment period. Data were analyzed with either a paired t-test, or the Wilcoxon signed rank test depending on parametric or non-parametric distribution. Comparing the data from before and after treatment showed the following findings: 1) a significant reduction in tender point counts, 2) significant reduction in WDI score, 3) significant reduction in SS score.
These data suggest a positive effect of a one month treatment with the AVACEN TES on

Fibromyalgia pain and related symptoms. Further studies in larger cohorts are warranted.

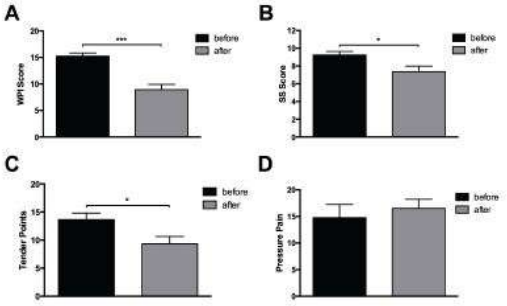


Figure 1: A significant difference was observed between timepoints (before and after) in the read-outs **(A)** Widespread Pain Index (WPI) score, **(B)** Symptom Severity (SS) score, and **(C)** Tender Points. No significant difference was observed in **(D)** Pressure Pain. Data were analyzed with either a paired t-test, or the Wilcoxon signed rank test depending on parametric or non-parametric distribution. All data are presented as Mean±SEM. Significance was assumed when *p<0.05. N (before) = 17; N (after) = 14.

Disclosures: T. Moeller-Bertram: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; AVACEN Inc.. **M.G. Kincaid:** None. **R. Booher:** None. **D. Garcia:** None. **L. Ky:** None. **J.M. Schilling:** None. **I. Strigo:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.06/BB2

Topic: C.14. Sensory Disorders

Support: KO1AT003883

R21AT004497

R03AT218317

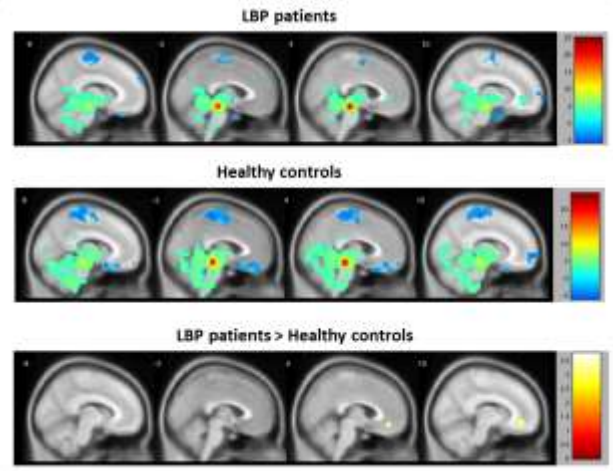
R01AT006364

R01AT005280

Title: Disrupted functional connectivity of the periaqueductal gray in chronic low back pain

Authors: *R. YU;
HMS-MGH, Charlestown, MA

Abstract: Introduction
Non-specific chronic low back pain is a common neurological disorder. The periaqueductal gray (PAG) plays a key role in the descending modulation of pain. In this study, we investigated brain resting state PAG functional connectivity (FC) differences between patients with chronic low back pain (cLBP) and matched healthy controls (HC).
Materials and Methods:
cLBP patients (age=36.1±9.9, 6 males) and age and gender matched healthy controls were scanned with a 3T Siemens Trio TIM scanner using a 32-channel head coil. Two resting-state BOLD fMRI scans were acquired with TR/TE=3000/30 ms, 3 mm isotropic resolution and 47 slices for 6.2 min each. Before and after each resting scan, study subjects were asked to rate the intensity of their LBP using a 0-10 scale.
PAG seed based functional connectivity (FC) analysis (PAG seed coordinate x=4, y=−26, z=−14, with 2mm radius) was performed to investigate the difference between the connectivity maps in the two groups ($P<0.05$, family wise corrected (FWE) after small volume correction). The correlation between whole brain FC and self-reported spontaneous pain intensity was also computed.
Results:
18 cLBP patients and XX matched controls completed the study. Data analysis showed increased FC between PAG and medial prefrontal cortex (mPFC) in cLBP patients compared with matched controls. In addition, we also found significant negative correlations between pain ratings and PAG-globus pallidus and PAG-orbitofrontal cortex FC. The cLBP illness duration was negatively correlated with PAG-insula FC.
Discussion / Conclusion:
These findings are in line with the impairments of the descending pain modulation reported in patients with cLBP. Our results provide evidence showing cLBP patients have abnormal FC in PAG centered pain modulation network during rest.



Disclosures: R. Yu: None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.07/BB3

Topic: C.14. Sensory Disorders

Support: NIH Grant R21AT004497

NIH Grant R01AT006364

NIH Grant R03AT218317

NIH Grant R01AT005280

NIH Grant PO1-AT002048

Title: Acupuncture modulates functional connectivity in knee osteoarthritis patients

Authors: X. CHEN[1], R. SPAETH[1], F. SONYA[1], D. SCARBOROUGH[2], R. EDWARDS[3], A. WASAN[3], R. GOLLUB[1], *J. KONG[1];
[1]Psychiatry, [2]Massachusetts Gen. Hosp., CHARLESTOWN, MA; [3]Brigham and Women's Hosp., Boston, MA

Abstract: Introduction
Recent advancesin brain imaging have contributed to our understanding of the neural activity associated with acupuncture treatment. However, most acupuncture studies to date have been conducted with healthy subjects in a single treatment session, which contrasts considerably with actual clinical practice. Here, we investigated neural activity in patients with knee osteoarthritis (OA) across severalacupuncture treatments.

Methods
OA patients were randomized to receive6 treatments of high dose, low dose, or sham acupuncture. 3T resting state functional magnetic resonance imaging scans were acquired pre and post the 1st, 3rd and 6th treatments.The Knee Injury and Osteoarthritis Outcome Score (KOOS) was used to measure clinical outcomes. Resting state data were analyzed using Independent Component Analysis (ICA) in FSL.Brain connectivity was compared between real (high and low dose) and sham acupuncture groups before and after the 4weeks oftreatment.

Results
Thirty patients (10/group) completed the study. Two sample t-tests indicated no significant

differences in clinical outcome between the high and low dose groups for any of the KOOS subscales. There was a significant interaction between acupuncture mode (real vs. sham) and time(baseline vs. endpoint) for pain ($F(1,28)=5.596$, $p=.025$) and function in sport ($F(1,27)=4.252$, $p=.049$).

ICA identified multiple resting networks with connectivity changes,including the executive control network (ECN) and somatosensory network (SN). In comparing the post-acupuncture scan from Treatment 6 to the pre-acupuncture scan from Treatment 1, the real acupuncture group demonstrated stronger ECN connectivity with rostral anterior cingulate gyrus (rACC) and medial prefrontal cortex (MPFC) than the sham group after acupuncture treatment. Interestingly, in comparing the pre-acupuncture scan from Treatment 1 to the pre-acupuncturescan from Treatment 6, the SN showed reduced connectivity in dorsal ACC (dACC) after real acupuncture treatment compared to the sham group. There is a gradual change inpre-treatment resting state connectivity across treatment sessions, which suggests an additive effect of real acupuncture in reducing connectivity in the dACC.

Conclusion

We found that real acupuncture can reduce the functional connectivity between the SN and dACC, a key region involved in the affective component of pain, and increase the functional connectivity between the ECN and rACC, a key region in the descending pain modulatory network. Our results suggest that acupuncture may achieve its known therapeutic effects on chronic pain through modulating functional connectivity.

Disclosures: X. Chen: None. **J. Kong:** None. **R. Spaeth:** None. **F. Sonya:** None. **D. Scarborough:** None. **R. Edwards:** None. **A. Wasan:** None. **R. Gollub:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.08/BB4

Topic: C.14. Sensory Disorders

Support: NIH K24 DA029262

Foundation for Anesthesia Education and Research

Title: Changes in low frequency oscillations in patients with neuropathic pain: A resting state fMRI study

Authors: *E. BAGARINAO, JR, I. CARROLL, N. CHATTERJEE, H. UNG, C. WANG, R. MOERICKE, S. MACKEY;
Stanford Univ., Palo Alto, CA

Abstract: Resting state functional magnetic resonance imaging is increasingly used to study neuropsychiatric disorders. In chronic pain, alterations in resting state networks, regions showing strong temporal correlation of low frequency oscillations (LFOs) across the brain, have been demonstrated in several studies [1]. In this work, we examined changes in the amplitude of these low frequency oscillations in patients with neuropathic pain (NP) compared to healthy controls (HCs). We hypothesized that neuropathic pain alters resting state LFOs in brain regions relevant to pain processing.

Resting state datasets from 28 participants (14 patients with NP and 14 age-matched HCs) were used in the analysis. The mean age is 43.43 (±14.22) years old for the NP group and 43.79 (±15.41) years old for the HC group. All participants gave informed consent prior to their participation and experimental protocols were approved by Stanford University Institutional Review Board. The functional datasets were preprocessed as follows: realigned relative to the first image in the series, co-registered to their respective anatomical image, normalized to MNI space, and smoothed using an 8mm full-width-at-half-maximum 3D Gaussian filter. The fractional amplitude of low frequency fluctuations (fALFF) within 0.01-0.08Hz was computed in all voxels, and then converted into a z-score. A two-sample t-test was used to compare the two groups. Contrast maps were cluster-corrected for multiple comparison using topological false discovery rate ($q < 0.05$).

Consistent with our hypothesis, patients with NP showed altered LFOs in regions implicated in pain modulation. Significant decreases in fALFF values were observed in right dorsal anterior cingulate cortex (dACC), left dorsolateral prefrontal cortex (dlPFC), and left anterior prefrontal cortex (aPFC) in patients with NP compared to HCs. These cingulo-frontal regions have been considered part of the descending pain modulatory pathway and play an important role in the control of pain perception. The left dlPFC, for example, has been shown to exert active control on pain perception by modulating the strength of coupling between midbrain and medial thalamus [2]. Changes in LFOs in these regions could signify alterations in this pain modulatory function in NP. Regions showing increases in fALFF values in the patient group include left occipital and lingual gyri. These findings further demonstrate the abnormalities in spontaneous brain activity observed in NP and provide additional insights into the mechanism of the functional changes involved.

[1] Baliki, et al., JNeurosci 28, 2008; Cauda, et al., PlosOne 4, 2009
[2] Lorenz, et al., Brain 126, 2003

Disclosures: E. Bagarinao: None. **I. Carroll:** None. **N. Chatterjee:** None. **H. Ung:** None. **C. Wang:** None. **R. Moericke:** None. **S. Mackey:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.09/BB5

Topic: C.14. Sensory Disorders

Support: NIH Grant NS046606

NCI Grant CA124787

Title: Evidence of decreased innervation density in multiple myeloma patients with subclinical peripheral neuropathy prior to chemotherapy

Authors: *A. KOSTURAKIS, Z. HE, H. ZHANG, P. M. DOUGHERTY;
Pain Res., MD Anderson Cancer Ctr., Houston, TX

Abstract: Preclinical studies show that chemotherapy-induced peripheral neuropathy could be due to the depletion of primary afferent sensory fibers after treatment. Recent studies indicate that disease-related processes of cancer may cause subclinical peripheral neuropathy in cancer patients prior to undergoing chemotherapy treatment. The goals of this study were 1.) to determine whether subclinical neuropathy is more prevalent than previously appreciated in a population of treatment naïve multiple myeloma (MM) patients using quantitative sensory testing (QST) analysis and 2.) to assess whether sensory deficits in patients is correlated with decreased innervation density of the extremities using non-invasive imaging. MM patients with no history of peripheral neuropathy underwent QST prior to chemotherapy. Skin temperature, sensorimotor function (grooved pegboard test) and detection thresholds for temperature, sharpness and low threshold mechanical stimuli (von Frey monofilaments and the Bumps detection test) were measured. Meissner's corpuscle (MC) density in the fingertips was assessed using *in vivo* laser reflectance confocal microscopy. Data from age-and sex-matched healthy volunteers was used as the control. Patients showed a high incidence of subclinical sensory deficits as demonstrated by higher thresholds of von Frey, Bumps, and warmth detection as compared to healthy volunteers. Patients also showed increases in cold pain, sensorimotor deficits, and higher overall neuropathy scores. MC density was significantly lower in patients compared with healthy volunteers and showed significant inverse correlation to Bumps detection threshold. Taken together, MM patients commonly present with sensory and sensorimotor deficits prior to undergoing chemotherapy treatment and these deficits appear to be correlated with a disease-related decrease in peripheral innervation density.

Disclosures: A. Kosturakis: None. **Z. He:** None. **H. Zhang:** None. **P.M. Dougherty:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.10/BB6

Topic: C.14. Sensory Disorders

Title: Multiple synaptic vesicle-associated proteins colocalize in mechanoreceptors and free nerve endings in skin

Authors: *B. D. MCADAMS[1], G. WENDELSCHAFER-CRABB[2], W. KENNEDY[2];
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Abstract: Synaptic vesicle proteins are critical for normal chemical neurotransmission. Synaptic proteins have been detected in cutaneous Merkel cells and in neuromuscular junctions in the peripheral nervous system (McAdams et al., 2011). In this study, we describe the distribution of presynaptic proteins in human cutaneous nerve fibers that are critical for sensory and neuroendocrine functions in skin. Peripheral nerve fibers were localized by immunostaining human skin with neural-specific PGP9.5/UCHL-1 and βIII-tubulin. In epidermal nerve fibers, SV2 and synaptophysin expression often was greatest in the most superficial endings in the stratum spinosum where the local gradient of extracellular calcium is highest. Although present in epidermal cells of the stratum basale, synaptotagmin 1 was not detected in the epidermal nerve fibers. Synaptotagmin-1 and VGLUT1, a presynaptic vesicular glutamate transporter, were, like SV2 and synaptophysin, strongly immunoreactive in two mechanoreceptive nerve structures of glabrous skin, the Meissner's corpuscles and Merkel cells. This suggests a neurosecretory function in these mechanoreceptors which includes glutamate release. Potential synaptic contacts in Merkel cells and Meissner's corpuscles were identified by dense clustering of mitochondria which colocalized with presynaptic protein immunostaining. Nerve fibers to sweat glands and blood vessels also co-expressed SV2, synaptophysin and synaptotagmin-1, but not with VGLUT1. Finally, while epidermal and dermal nerve fibers immunostained for the scaffolding protein ankyrinB, as demonstrated previously (Engelhardt et al., 2012), resident Schwann cells and adjacent nerve fibers of the Meissner's corpuscle were immunopositive for ankyrinB. These results confirm that synaptic vesicle proteins are present in cutaneous nerve fibers including in mechanoreceptors. A potential autocrine or paracrine role in neuromodulatory signaling of these cutaneous fibers may explain the localization of these synaptic proteins in cutaneous nerve. Future research on the function of these proteins in cutaneous neurons could provide clinically relevant biomarkers for molecular diagnosis of peripheral sensory neuropathies, such as those afflicting patients with diabetes and cancer.

Disclosures: B.D. McAdams: None. **G. Wendelschafer-crabb:** None. **W. Kennedy:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.11/BB7

Topic: C.14. Sensory Disorders

Support: Swedish Rheumatism Association

Title: Fibromyalgia patients and healthy controls show differing cortical activation patterns during the stroop task: An fMRI study

Authors: *S. MARTINSEN[1], J. BERREBI[1], P. FLODIN[1], I. VILEVICIUTE-LJUNGAR[2], M. LÖFGREN[2], M. INGVAR[1], P. FRANSSON[1], E. KOSEK[1];
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Abstract: Introduction
In response to painful stimulation, a network of areas in the brain activate, including the sensory cortices, insula, thalamus and the anterior cingulate cortex (ACC). The level of activity in the ACC has been found to differ between healthy controls and chronic pain patients during nociceptive stimulation (Jensen et al. 2009), specifically; fMRI studies have shown that fibromyalgia (FM) patients show less activity in the rostral ACC (rACC) during painful stimulation. The ACC has also been found to be involved in various aspects of higher order cognitive processes, something of which FM patients report having difficulty with. Previous studies using the Stroop task (a measure of response inhibition) have found that low performers show greater activity in the ACC compared to high performers (Floden et al. 2010). Presumably this could reflect a differential degree of involvement of the ACC linked to degree of cognitive work that is required to perform the task at hand. Using the Stroop task we wanted to investigate the role of the ACC in FM for a cognitively demanding task. We hypothesized that the patient group would engage their ACC more strongly than healthy controls. Holding true, this would suggest that the ACC dysfunction seen in FM patients might be coupled to chronic pain.
Methods
15 female FM patients and 18 female healthy controls were included in the study. Participants underwent functional magnetic resonance imaging (fMRI) during which they performed two 10 minutes sessions of an event related version of the Stroop paradigm (red green and yellow written in congruent or incongruent color). Participants were given a response box with buttons

matching the colors used in the task, and they were instructed to respond to the color of the text and not to the meaning of the word presented. Image pre-processing and statistical analysis were performed in SPM8.
Results
At the within-group level we found similar activation patterns for both groups, including the inferior frontal gyrus, ACC, and superior parietal lobule. When comparing activation patterns across groups, we found that FM patients showed higher activation in the ACC (x -2, y 26, z 40, peak T value 3.25, $p<0.001$ uncorrected).
Conclusions
In line with our hypothesis, a between-group comparison showed that the FM group activated the ACC more strongly than the HC group for the Stoop task.
We interpret the stronger ACC activation for patients relative to healthy controls as an additive effect in the anterior cingulate cortex during the Stoop task. Presumably, this is related to the negative effects of chronic pain on cognitive function.

Disclosures: S. Martinsen: None. **J. Berrebi:** None. **P. Flodin:** None. **I. Vileviciute-Ljungar:** None. **M. Löfgren:** None. **M. Ingvar:** None. **P. Fransson:** None. **E. Kosek:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.12/BB8

Topic: C.14. Sensory Disorders

Support: NIH ARRA grant RC1 MH088678

Duke Institute for Brain Sciences Research Incubator Award

Title: Developmental trajectories of insula volume changes in adolescent anorexia nervosa

Authors: *P. A. KRAGEL, J. WANG, R. VALDOVINOS, N. L. ZUCKER, K. S. LABAR; Duke Univ., Durham, NC

Abstract: Adolescence is a key developmental period for functional and structural changes in brain regions associated with interoception and self-referential processes, including those related to psychosomatic illness. Anorexia nervosa (AN) is an eating disorder that develops primarily in adolescence and is characterized in part by dangerous food restriction, heightened visceral sensitivity, and altered interoception. Neurobiological models of interoceptive awareness characterize a successive integration of information within the insula, starting with a primary

representation of interoceptive state in the posterior insula and progressively forming a complex unified representation of the self in the anterior insula. We sought to examine differences in developmental trajectories of anterior and posterior insula gray matter volume in female adolescents with AN (N = 22, M_{age} = 17.15) and age matched healthy controls (N = 23, M_{age} = 17.14). Anatomical scans were performed using a FSPGR BRAVO sequence acquired in the axial plane, with an image matrix of 256 x 256 and 1-mm isotropic voxels. Reconstructed images were manually segmented using ITK-SNAP software, separately demarcating anterior and posterior insula (bounded by the central insular sulcus) within each hemisphere. Following segmentation, volumes were normalized relative to the total intracranial volume for each subject. Changes in relative volume were assessed using an analysis of covariance including clinical group, linear age, and nonlinear age, along with relevant interaction terms, as regressors in the model. The analysis revealed significant linear and nonlinear effects of age for both the left and right anterior insula, showing an asymptotic reduction in relative volume independent of clinical status. Additionally, significant interactions between clinical group and linear as well as nonlinear age were evident in the right posterior insula, indicating a relative increase in volume during mid-adolescence for clinical compared to control subjects. These results support neurobiological models implicating insular dysfunction in AN as well as models emphasizing mid-adolescence as a key inflection point for structural changes in social and affective brain function that may confer vulnerability to psychiatric illness. Furthermore, the findings highlight a potential substrate for alterations in visceral sensitivity and interoceptive processing commonly associated with AN.

Disclosures: P.A. Kragel: None. **J. Wang:** None. **R. Valdovinos:** None. **N.L. Zucker:** None. **K.S. LaBar:** None.

Poster

539. **Somatosensory and Pain: Human Subjects**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 539.13/BB9

Topic: C.14. Sensory Disorders

Support: NIH/NIDCR R21 DE018561

R01 DE019796

Title: ATP and its receptor P2X2 and P2X3 in oral cancer induced pain

Authors: *Y. YE[1], K. ONO[2], D. BERNABE[2], C. VIET[2], J. DOLAN[2], A. FORD[3], B. SCHMIDT[2];

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Abstract: Extracellular ATP is an endogenous fast-acting neurotransmitter and plays a key role in pain signaling. ATP signals pain through peripheral P2X2 and P2X3 receptors in animal models of inflammatory and neuropathic pain. The role of ATP and its receptor P2X2 and P2X3 in oral cancer induced pain is less clear. In the present study, we used a translational approach studying both human and animal models of oral cancer in parallel. ATP concentration was measured using HPLC in human oral squamous cell (SCC) carcinoma tissues. Pain levels were measured using a validated oral cancer pain questionnaire. A strong positive correlation of ATP concentration in the tumor and levels of pain in oral cancer patients were found. We also found that human SCC is densely innervated by sensory fibers expressing both P2X2 and P2X3 receptors. Our calcium imaging and patch clamping data showed that SCC releases ATP that can directly activate trigeminal ganglion (TG) neurons expressing P2X2 and P2X3 receptors. Using three different animal models of SCC, we demonstrated that selective P2X3 and P2X2/3 antagonists significantly reduced SCC-induced pain as measured by an electronic von Frey and a gnawing assay. Co-culture with SCC increased expression of both P2X2 and P2X3 receptors in TG neurons, which is modulated by nerve growth factor (NGF) in the SCC microenvironment. Our results demonstrate that ATP and its receptors P2X2 and P2X3 play significant roles in oral cancer pain both in human and animal models.

Disclosures: Y. Ye: None. **K. Ono:** None. **D. Bernabe:** None. **C. Viet:** None. **J. Dolan:** None. **A. Ford:** None. **B. Schmidt:** None.**Poster**

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.01/BB10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Ontario Brain Institute to the Canadian Biomarker Integration Network for Depression

Buchan Family Foundation

Toronto General and Western Hospital Foundation

Title: Baseline and change resting-state functional correlates of rTMS of the DMPFC for medically refractory anorexia and bulimia nervosa

Authors: *K. DUNLOP[1], T. SALOMONS[3], N. BAKKER[4], J. GERACI[3], P. GIACCOBE[3,5], M. OLMSTED[2], P. COLTON[2], B. WOODSIDE[2], J. DOWNAR[2,5,3];

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Abstract: Transcranial magnetic stimulation (rTMS) has been recently suggested as a potential treatment for anorexia and bulimia nervosa. Previous research has shown modest efficacy with rTMS for eating disorders using the conventional dorsolateral prefrontal cortex target. However, recent neuroimaging research suggests that the dorsomedial prefrontal cortex (DMPFC) is a key region for impulse control and behavior regulation, including bingeing and purging behaviour. Thus, the DMPFC may be a potential rTMS target for the treatment of eating disorders. 20 patients with treatment refractory anorexia and bulimia nervosa underwent 20 sessions of open-label, add-on rTMS to the DMPFC (10 Hz bilateral stimulation, 120% resting motor threshold). Clinical measures, structural and resting-state scans were obtained before and after treatment. Analysis was performed in FSL. Data was first pre-processed (motion corrected, spatially smoothed, regression of global, white matter and cerebrospinal signal, bandpass filtered). Following pre-processing seed-based resting state analysis was performed for *a priori* regions-of-interest (bilateral ventral striatum [BVS] and subgenual cortex [sgACC]) and a region in proximity to the area stimulated (DMPFC). Purges/week change (baseline-week 4) categorized subjects into a responder (improvement over 50%) or a non-responder (improvement under 50%). This was used as a regressor for the following group-level analysis: 1) baseline connectivity, and 2) connectivity change. Clinical results showed that purging from baseline to week 4 improved from 25.3±38.8 episodes/week to 17.3±19.2 episodes/week. Baseline DMPFC connectivity was not significantly correlated to response. However, for the BVS seed, high pre-treatment functional connectivity to the precuneus and posterior cingulate cortex (PCC), and for the sgACC seed, high pre-treatment functional connectivity to the precuneus and PCC and low functional connectivity to the right hippocampus and amygdala and midbrain Raphé nuclei, were significantly correlated to clinical response. sgACC connectivity change was not significantly correlated to treatment response. It was found that decreased BVS connectivity to the precuneus and PCC and decreased DMPFC connectivity to the frontopolar cortex were significantly correlated to clinical response. In general, this preliminary study showed altered connectivity in midbrain serotonergic structures and corticostriatal and corticocortical pathways previously implicated in emotion regulation and in the pathophysiology of disordered eating behaviour. A randomized control trial as a next step may be warranted.

Disclosures: K. Dunlop: None. **T. Salomons:** None. **N. Bakker:** None. **J. Geraci:** None. **P. Giaccobe:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain Cells Inc., Clera, GSK, St. Jude Medical, Atra-Zeneca, BMS. F. Consulting Fees (e.g., advisory boards); Eli Lilly. **M. Olmsted:** None. **P. Colton:** None. **B. Woodside:** None. **J. Downar:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and

pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Lundbuck Canada.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.02/BB11

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: EEG source analysis in depressed patients treated with left prefrontal 5Hz transcranial magnetic stimulation

Authors: *J. J. GONZÁLEZ-OLVERA[1], J. RICARDO-GARCELL[2], M. L. GARCÍA-ANAYA[3,2], E. M. MIRANDA-TERRÉS[3], E. REYES-ZAMORANO[3];
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Abstract: Transcranial Magnetic Stimulation (rTMS) is a technique that allows non invasive electrical stimulation of the cortex with few side effects. It has been proposed an antidepressant effect when rTMS is delivered over prefrontal dosrolateral cortex (DLPFC) ≥ 5Hz. Quantitative EEG studies have shown increases in alpha and theta power bands as well as frontal interhemispheric asymmetries in most recordings from depressed patients. rTMS over left DLPFC at 5Hz involve a safer and more tolerable procedure however its neurophysiological correlates has not been explored using EEG source analysis. The aim of this research was to study changes in EEG sources using VARETA method in a group of patients with major depressive disorder (MDD) treated with 5 Hz rTMS over left DLPFC as single or combined treatment with escitalopram. Methods: 18 patients with DSM-IV MDD diagnosis without treatment for the current episode were included. Subjects were randomly assigned to one of two groups: A) rTMS + escitalopram 10mg, n=9; B) rTMS + placebo, n=9. Subjects received 15 sessions of rTMS on a daily basis. In order to compare changes in EEG sources two recordings were obtained, prior and after treatment. HDRS, BDI and HARD were used for clinical assessments. Results: All patients of group A and 8 patients of group B showed response to treatment (considered as a reduction of 50% in HDRS score). An increase in absolute power at 9.37 Hz and 10.17 Hz in temporal and postcentral gyrus on the left hemisphere was found in group B, on the contrary absolute power those frequencies were decreased in the same regions for group A. In addition an increased power in beta band frequencies were observed in both

hemispheres for group A. Conclusion: Increases in alpha band could be the hallmark of the 5 Hz rTMS, but it could be reduced by escitalopram. Besides, increases observed in beta band for group A could be related to escitalopram effect.

Disclosures: J.J. González-Olvera: None. **J. Ricardo-Garcell:** None. **M.L. García-Anaya:** None. **E.M. Miranda-Terrés:** None. **E. Reyes-Zamorano:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.03/BB12

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: CNPq

FAPERGS

FINEP

Title: Study of association between brain-derived neurotrophic fator polymorphism (BDNF Val66Met) and suicide risk in a population-based study

Authors: *G. GHISLENI[1], F. P. MOREIRA[1], F. N. KAUFMANN[1], J. D. FABIÃO[1], E. SCHUCH[1], R. A. SILVA[1], D. CRISPIM[2], D. R. LARA[3], M. P. KASTER[1];
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Abstract: Brain-derived neurotrophic fator (BDNF) is a member of the neurotrophin growth factor family and mediates neural plasticity, mood, different behaviors, and stress response. A functional BDNF polymorphism (Val66Met) was associated with depression and suicidal behavior. The goal of the present study was evaluate the association between BDNF Val66Met variants and suicide risk in a population-based study. This study was carried out with 482 subjects of the South of Brazil evaluated with a structured diagnostic interview - Mini-International Neuropsychiatry Interview (M.I.N.I; Sheehan et al., 1998). The BDNF Val66Met polymorphism was genotyped by qPCR from 386 control and 96 subjects with suicide risk and the groups were subdivided according to gender. The Val66Met genotypes were in agreement with those predicted by the HWE. All participants gave written informed consent and the ethic committee approved the study. The studied population have mean age of 26.33±5.1 years, 62.4% are women and 80.2% are caucasian. Between subjects with suicide risk 83.2% (n=79) have

major depression and 43.2% (n=41) bipolar disorder (p=0.001). Frequency of BDNF Val66Met genotypes was different between gender [24.9% (n=75) Val/Met-Met/Met in women vs 34.8% (n=63) Val/Met-Met/Met in men; p=0.026], but did not differ for age (26.28±5.1 Val/Val vs 26.43±5.1 Val/Met-Met/Met; p=0.767) and ethnicity [30.2% (n=117) Val/Met-Met/Met in caucasian vs 22.3% (n=21) Val/Met-Met/Met in non-caucasian; p=0.169]. All included suicide risk subjects and the control groups were not different for Val66Met genotypes frequency [32.3% (n=31) Val/Met- Met/Met vs 27.7% (n=107) Val/Met-Met/Met; respectively, p=0.447). The BDNF Val66Met also not differ between suicide risk subjects and the control groups for the women [30.0% (n=21) Val/Met / Met/Met vs 23.4% (n=54) Val/Met, Met/Met; respectively, p=0.335], and male groups [38.5% (n=10) Val/Met / Met/Met vs 34.2% (n=53) Val/Met, Met/Met; respectively, p=0.841]. The combined Met/Met and Met/Val was not significantly associated with suicide risk in the population studied, suggesting that BDNF Val66Met is not predictor of risk for suicide attempt in subjects with mood disorder.

Disclosures: G. Ghisleni: A. Employment/Salary (full or part-time):; Universidade Católica de Pelotas. **F.P. Moreira:** None. **F.N. Kaufmann:** None. **J.D. Fabião:** None. **E. Schuch:** None. **R.A. Silva:** None. **D. Crispim:** None. **D.R. Lara:** None. **M.P. Kaster:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.04/CC1

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: CNPq

FAPEG

Title: Evaluation of CYP2C19 genotype influence on dose-response to treatment by escitalopram

Authors: *P. C. GHEDINI, R. B. BRITO, K. S. A. SILVEIRA;
Federal Univ. of Goiás, Goiânia, Brazil

Abstract: The CYP2C19 gene presents variation sites that may affect the pharmacokinetics of several drugs of clinical importance, including antidepressant medications as escitalopram. In this study, we investigate the impact of CYP2C19 genotype on escitalopram treatment in patients with remission of depressive symptoms taking this antidepressant at oral doses of 10, 15 or 20 mg/day and with an average use of 2.5 years. DNA was extracted from blood samples obtained

from 22 patients (6 males and 16 females, aged 22-66 years) and CYP2C19*2 and *3 mutations were evaluated by PCR-RFLP. All experiments were approved by the local Ethics in Research Committee (Protocol CEP/UFG 204/2009). Two genotypes were found in the present samples, including 16 subjects (72.7%) with no mutated alleles (*1/*1) and 6 (27.3 %) with one mutated allele (*1/*2). No sample was found carrying two CYP2C19 mutated alleles (*2/*2) and *3 allele was not found in the present series. The dose of 15 mg/day escitalopram was the most frequent in patients carriers of loss-of-function *2 allele (4; 66.8%), whereas the doses of 10 and 20 mg/day had the same distribution (1; 16.6%). These results were not different when compared to non-carriers of *2: 62.6% (10), 18.7% (3) and 18.7% (3) for doses of 15, 10 and 20 mg/day, respectively. The influence of sex and age was not observed. Despite several limitations including the lack of serum drug levels and the sample size of patients, this preliminary study suggested that there isn't influence of CYP2C19*2 on dose-response to chronic treatment by escitalopram.

Disclosures: P.C. Ghedini: None. **R.B. Brito:** None. **K.S.A. Silveira:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.05/CC2

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH085734

Title: Altered functional connectivity of subgenual anterior cingulate cortex during negative emotion processing in adolescents with depression

Authors: *T. C. HO[1], G. YANG[2], J. WU[1], G. A. FONZO[3], C. G. CONNOLLY[1], M. CHAN[2], N. HOANG[1], A. N. SIMMONS[2,4], T. YANG[1];
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Abstract: Major depressive disorder (MDD) typically begins during adolescence and confers a high risk of recurrence in adulthood. Recent evidence suggests that adult MDD is partially characterized by dramatic alterations in the functional connectivity of brain regions involved in emotion processing (Stuhrmann et al., 2011). Thus, examining the functional connectivity of these areas in adolescent MDD could elucidate the etiology and progression of this disorder within the context of brain changes that occur during this sensitive period of development. The

subgenual anterior cingulate cortex (sgACC) and its connected circuitry are heavily implicated in emotion function and as such, may be more susceptible to changes caused by MDD during adolescence (Cullen et al., 2009; Davey et al., 2012). However, few studies have investigated functional connectivity of the sgACC in depressed youth during the processing of emotionally negative stimuli. Using functional magnetic resonance imaging (fMRI), we scanned 45 medication-naïve adolescents (ages 13-17 yrs): 19 MDD and 26 healthy controls (HCL). Depression severity was measured with the Beck Depression Inventory (BDI) and the Children's Depression Inventory (CDI). Subjects performed a gender recognition task of faces exhibiting varying degrees of fear (strong, moderate, and weak) during scanning. We defined seeds in bilateral sgACC and assessed functional connectivity using the psychophysiological interaction method (Friston et al., 1997). First, we found significantly increased functional connectivity between the sgACC and the insula during viewing of strong versus weak fear stimuli in the MDD relative to HCL group. Second, we found decreased functional connectivity between the sgACC and dorsal frontal regions and between the sgACC and dorsal ACC during viewing of strong versus weak fear stimuli in the MDD relative to HCL group. Importantly, the strength of connectivity between the sgACC and dorsal ACC in our MDD group correlated positively with BDI and CDI scores. Our observation of elevated connectivity between the sgACC and insula may be due to increased activation in these structures resulting from enhanced processing of negative stimuli. Likewise, our finding of diminished connectivity between sgACC and cingulate and frontal areas may also reflect decreased regulation of cingulate activation in response to negative emotional stimuli. Similar results have also been reported in adults with depression (Stuhrmann et al., 2011). Our study therefore offers preliminary evidence that aberrant connectivity observed in adolescent-onset depression may affect connectivity patterns seen in adult depression.

Disclosures: T.C. Ho: None. **G. Yang:** None. **J. Wu:** None. **G.A. Fonzo:** None. **C.G. Connolly:** None. **M. Chan:** None. **N. Hoang:** None. **A.N. Simmons:** None. **T. Yang:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.06/CC3

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Ontario Brain Institute to the Canadian Biomarker Integration Network for Depression

Buchan Family Foundation

Toronto General and Western Hospital Foundation

Title: Neuroimaging predictors of treatment response to intermittent theta-burst repetitive transcranial magnetic stimulation of the dorsomedial prefrontal cortex in refractory depression

Authors: *N. BAKKER, IV[1], J. DOWNAR[5,2,3], P. GIACOBBE[5,2], T. SALOMONS[5], J. GERACI[5], K. DUNLOP[4], D. BLUMBERGER[2,6], Z. J. DASKALAKIS[2,3,6], S. KENNEDY[2,5,3], A. FLINT[2,5,3];
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Abstract: The conventional target of repetitive transcranial magnetic stimulation (rTMS) for refractory major depressive disorder (MDD) has been the dorsolateral prefrontal cortex (DLPFC), but convergent evidence from lesion, stimulation, and neuroimaging studies suggests that the dorsomedial prefrontal cortex (DMPFC) might serve a more central function in emotion regulation. We recently targeted the DMPFC with 10 Hz rTMS in an open-label case series of 47 patients with MDD and found that it was safe, tolerable, and effective, with 51% of patients responding and 43% of patients achieving remission. We also observed a bimodal response pattern, such that patients either experienced minimal or marked improvement. Before treatment, non-responders showed disrupted hedonic function and abnormal connectivity in reward circuity, whereas responders showed preserved hedonic function and normal connectivity in reward circuitry. Although promising, the major limitation with 10 Hz rTMS is the long duration of each treatment session, requiring 40 minutes per day over 20-30 sessions. Drawing on recent studies suggesting that intermittent theta-burst (iTBS) protocols can achieve stronger effects in less time, we targeted the DMPFC with iTBS for 7 minutes per day over 20-30 sessions in an open-label case series of 47 patients with MDD. Despite the significant reduction in treatment time, we found that iTBS of the DMPFC was just as effective as 10 Hz rTMS, with 49% of patients responding and 43% of patients achieving remission. We also collected pre-treatment resting state functional neuroimaging data, which were analyzed using graph theoretical measures and will be discussed in relation to our previous findings linking non-response to anhedonia and abnormal reward circuitry. These results emphasize the utility of iTBS rTMS of the dmPFC for treating refractory MDD and suggest that hedonic functioning and reward circuitry could help predict treatment response.

Disclosures: N. Bakker: None. **J. Downar:** Other; Lundbeck Canada. **P. Giacobbe:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Brain Cells Inc., Clera, GSK, St. Jude Medical. F. Consulting Fees (e.g., advisory boards); Eli Lilly. Other; Astra-Zeneca, BMS, Pfizer, Eli Lilly, St. Jude Medical. **T. Salomons:** None. **J. Geraci:** None. **K. Dunlop:** None. **D. Blumberger:** None. **Z.J. Daskalakis:** None. **S. Kennedy:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Astra Zeneca, Bristol-Myers Squibb, Brain Cells Inc., Clera, Eli Lilly, GlaxoSmithKline, Lundbeck, St Jude

Medical. F. Consulting Fees (e.g., advisory boards); Astra Zeneca, Eli Lilly, Lundbeck, Pfizer, Servier, St Jude Medical, Spimaco. Other; Servier, Eli Lilly, Spimaco, Bristol-Myers Squibb, Astra Zeneca, Lundbeck. **A. Flint:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lundbeck Canada. Other; Janssen-Ortho, Lundbeck Canada, Pfizer Canada.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.07/CC4

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: DOD W81XWH0710244

Title: A common snp variant in FKBP5 is associated with global reductions in levels of mediodorsal thalamic miRNAs in controls and schizophrenia, but not depression

Authors: *K. A. YOUNG, E. S. CARTER, D. PAPPALARDO CARTER;
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Abstract: FKBP5 encodes an immunophilin which plays a role in immunoregulation, protein folding/ trafficking, calcineurin inhibition, progesterone receptor and heat shock protein complexes and glucocorticoid receptor (GR) regulation. Genetic alterations in FKBP5, including the common SNP variant rs1360780, have been repeatedly implicated as a risk factor for mood disorders. We have previously found genetic influences on structural alterations in the mediodorsal thalamic nucleus in major depression (MDD) and schizophrenia (SKZ), which is of interest because this nucleus extends robust projections to the frontal cortex mediating aspects of frontal cortex dysfunction in these two conditions. MicroRNAs (MiRNAs or miRs) are small non-coding RNAs which modify gene expression and other processes, usually by regulating protein translation. We investigated the possibility that the intronic SNP rs1360780 influences MDD susceptibility though alterations in miRs by assaying levels of 750 human miRs in the mediodorsal thalamic nucleus with RT-PCR panels (Exiqon). In the cohort as a whole, we observed that the common risk genotype for FKBP5 (CT) was associated with a global average 4-fold reduction in 737 out of 750 miRs. 298 of the reductions were significant at a $p<0.05$ level, while no MiRs were significantly elevated. Randomly chosen miRs (hsu-miR-584, -7693-p, -877*, -93, -933, -937) with p values approaching, or less than $p<0.01$ were studied in more detail. The four-fold reduction with CT genotype remained significant after co-varying for diagnosis, age, gender, PMI and pH, and in the two psychiatric subgroups, suicide was not

significantly associated with miR levels. Although we did not observe a main effect of diagnosis on miR levels, we did observe an interaction, with miR reductions associated with the CT genotype being accentuated in controls and SKZ to an average 7-fold reduction, contrasting with a loss of the CT genotype effect in the MDD group (average fold change = - 0.15). Although the mechanisms responsible for these observations remain obscure, it is known that glucocorticoids can suppress expression of key miR processing enzymes including Drosha, Dicer, and DGCR8/Pasha. By sensitizing GR activity or altering its nuclear translocation, the FKBP5 rs1360780 "T" allele may contribute to reductions in miR processing activity, instigating a global down-regulation of the miRnome. Cells with a globally repressed microRNA regulation system may be at higher risk for malfunction under stress, contributing to a neurobiological basis for susceptibility to a variety of stress related disorders such as MDD.

Disclosures: K.A. Young: None. **E.S. Carter:** None. **D. Pappalardo Carter:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.08/CC5

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Neural predictors of antidepressant treatment response to Quetiapine XR and Citalopram in Major Depressive Disorder

Authors: *A. BURGESS[1], R. WHITE[1], F. CORTESE[2], B. GOODYEAR[2], A. PANICKER[1], A. KARNES[1], K. ROY[1], V. DIWADKAR[1], R. RAMMASUBBU[2];
[1]Psychiatry and Behavioral Neurosci., Wayne State Univ. Sch. of Med., Detroit, MI; [2]Univ. of Calgary, Calgary, AB, Canada

Abstract: Background: Clinical response to antidepressant treatment is varied and unpredictable in patients with major depressive disorder (MDD). Imaging studies have shown general neural predictors of clinical improvement to antidepressants (Pizzagalli 2011). However, identifying differential predictors of clinical response to antidepressants with different pharamacological profiles may help match patients with appropriate anti-depressants early in treatment. Here we investigated general and specific neural predictors of antidepressant treatment response to citalopram and quetiapine XR treatment in MDD.
Methods: During fMRI (3T), 46 subjects with MDD participated in a picture-matching task (Hariri et al., 2003) during which subjects' matched pairs of faces (or geometric objects) among triads of stimuli. The event-related design was analyzed in SPM8 using standard methods. Pre-

trial fMRI responses were compared (based on end outcome; 8-week) between clinically improved and non-improved groups. 2nd level random effects analyses of fMRI data assessed activity-related differences for both positive and negative valence classes. Analyses were thresholded to a depression and treatment recovery neural circuit consisting of the amygdala, dorsal ACC, subgenual ACC, precuneus, orbito-frontal, and dorsolateral frontal cortex.
Results: Responders to antidepressant treatment showed increased pre-treatment activation in frontolimbic regions including the dorsal nexus, dorsal prefrontal cortex, ACC, orbitofrontal cortex and amygdala (Figure 1).
Conclusions: These findings confirm that pre-treatment anterior cingulate activity to negative emotions may represent generic predictor of clinical improvement to antidepressants. Future studies should examine whether functional interactions of ACC with other regions implicated in emotional regulation or depression differentially predict antidepressant response.

Disclosures: A. Burgess: None. **R. White:** None. **F. Cortese:** None. **B. Goodyear:** None. **A. Panicker:** None. **A. Karnes:** None. **K. Roy:** None. **V. Diwadkar:** None. **R. Rammasubbu:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.09/CC6

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Office of Public Health

Title: The Additive Impact of Clinical Depression on white matter abnormalities in veterans with Co-morbid PTSD &Traumatic Brain Injury: A diffusion tensor imaging study

Authors: L. ISAAC[1], K. MAIN[1], S. SOMAN[1], J. KONG[1], I. GOTLIB[2], A. FURST[1], J. ASHFORD[1], M. ADAMSON[1], *P. J. BAYLEY[3,1];
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Abstract: A significant proportion of military personnel deployed in support of Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) have been exposed to war-zone events that are potentially associated with traumatic brain injury (TBI), major depressive disorder (MDD) and posttraumatic stress disorder (PTSD). The co-occurrence of TBI, PTSD,

and MDD in returning veterans and the symptom overlap among the three disorders has fueled both research and clinical interest in elucidating the unique and shared effects of such injuries. The purpose of this study was to test the hypothesis that white matter abnormalities are present in association fibers of the uncinate fasciculus (UF), a key fronto-temporal tract involved in mood regulation, and cingulum bundle (CB), a tract that connects to the hippocampus and is involved in memory integration to other parts of the brain. Both the UF and CB tracts, considered part of the limbic system, are posited to be involved in emotion processing, attention, and memory, all of which have been implicated in depression. We performed diffusion tensor imaging on 25 patients with a combination of PTSD, TBI and MDD and 20 patients with PTSD and TBI without MDD matched on age (M = 44.65, SD = 12.0), gender distribution (88% male), education (M = 13.96 , SD = 2.28), and deployment duration in years (M = 7.9, SD = 3.5). We measured the impact of clinical depression on white matter integrity by comparing these two patient groups, who were differentiated only with respect to the presence or absence of MDD. A hierarchical logistic regression model comparing fractional anisotropy (FA) values revealed that after controlling for age, the model correctly classified and distinguished 86.2% of patients who had PTSD, TBI and MDD from the patient group with PTSD and TBI alone. However, the model was able to correctly classify only 61.3% of the patients who had only PTSD and TBI. The regression coefficients indicated that both the right CB ($\beta = 21.04$, $p = .05$) and the left UF ($\beta = 32.05$, $p = .045$) were significant predictors of group assignment. Both the left UF and the right CB had significantly lower FA values in the group with MDD. Our findings provide new evidence of microstructural changes in white matter in Veterans with clinical depression. These results complement those obtained in previous work on depression and support the hypothesis that the disruption of cortical-subcortical circuit integrity is involved in the etiology of MDD. Thus, a detailed examination of the tracts that connect these regions has implications for the management, treatment, and pathophysiology of these commonly observed co-morbidities in the veteran population.

Disclosures: L. Isaac: None. **K. Main:** None. **S. Soman:** None. **J. Kong:** None. **I. Gotlib:** None. **A. Furst:** None. **J. Ashford:** None. **M. Adamson:** None. **P.J. Bayley:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.10/CC7

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: R01 MH46848

R01 MH80109

R01 DA09397

Title: Cerebrospinal fluid glutamate concentration correlates with impulsive aggression in human subjects

Authors: E. F. COCCARO, R. LEE, *P. VEZINA;
Dept Psychiatry and Behavioral Neurosci., The Univ. of Chicago, Chicago, IL

Abstract: Neurochemical studies have pointed to a modulatory role in human aggression for various central neurotransmitters. Some (e.g., serotonin) appear to play an inhibitory role, while others appear to play a facilitator role. While recent animal studies of glutaminergic activity suggest a facilitator role for central glutamate in the modulation of aggression, no human studies of central glutaminergic indices have yet been reported regarding aggression. Basal lumbar cerebrospinal fluid (CSF) was obtained from 38 physically healthy subjects with DSM-IV Personality Disorder (PD: n = 28) and from Healthy Volunteers (HV: n = 10) and assayed for glutamate and other neurotransmitters in CSF and correlated with measures of aggression and impulsivity. CSF glutamate levels did not differ between the PD and HV subjects but did directly correlate with composite measures of both aggression and impulsivity and a composite measure of impulsive aggression in both groups. These data suggest a positive relationship between CSF glutamate levels and measures of impulsive aggression in human subjects. Thus, glutamate function may contribute to the complex central neuromodulation of impulsive aggression in human subjects.
Supported by NIH grants R01 MH46848, R01 MH80109 (Coccaro), and R01 DA09397 (Vezina).

Disclosures: E.F. Coccaro: Other; on Scientific Advisory Board of Azevan Pharmaceuticals, Inc. **R. Lee:** Other; Past recipient of a research grant from Azevan Pharmaceuticals, Inc.. **P. Vezina:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.11/CC8

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Medtronic Inc

Title: Discharge rates of neurons in the posterior hypothalamus region in Sotos syndrome

Authors: *W. D. HUTCHISON[1], R. MICIELI[2], N. TRUJILLO[3], A. LOPEZ RIOS[4];
[1]Div. Neurosurg., Toronto Western Hosp., Toronto, ON, Canada; [2]Physiol., Univ. of Toronto, Toronto, ON, Canada; [3]Neurosci., Univ. of Antioquia, Medellin, Colombia; [4]Centros Especializados Rionegro, Hosp. San Vicente Fundacion, Medellin, Colombia

Abstract: Deep brain stimulation (DBS) of the posterior hypothalamus (PH) has been reported to be effective for aggressive behaviour in a number of isolated case studies. Sotos syndrome is rare genetic (NSD1- nuclear set domain) disorder leading to cerebral gigantism and neuropsychiatric problems in children, including aggression. Single unit neuronal recordings in the human PH have been reported in cluster headache (Sani et al. Neurosurg 64: 161, 2009), but not for Sotos syndrome. We report on the properties of ongoing neuronal discharge in bilateral trajectories targeting PH in a young female with Sotos syndrome. Initial target coordinates, determined by magnetic resonance imaging stereotactic localization, were 2 mm lateral, 3 mm posterior, and 5 mm inferior to the midpoint of the anterior commissure- posterior commissure line. Two microelectrodes were driven down the central and lateral guide tubes of the Guideline 4000 system (Frederick Haer) starting at 15 mm above the tentative target in PH under local anesthetic only. Single units were discriminated off-line by template matching using Spike2 software (CED). Average firing rates and oscillatory activity were compared for three regions, ventral thalamus (n=12 units), PH region (n=15) and red nucleus (n = 4). The ventral thalamus appeared to have two populations of neurons, one with a high firing rate (16.5 +/- 7.0 Hz n=6) and one with a low firing rate (5.1 +/- 3.1 Hz, n=6). Most of the PH region units had a slow, irregular discharge (5.7 +/- 3.4 Hz, n=10) but some units also had a higher rate (19.3 +/- 5.8 Hz, n=5). The mean discharge rate of PH units was not significantly different overall than that of ventral thalamus. Red nucleus neurons had higher firing rates (25.9 +/- 9.7 Hz, n=4) and regular firing with periods of oscillatory activity in the beta range (15 - 20 Hz). Oscillatory activity was not evident in the PH or ventral thalamus. Early observation indicated that the patient's aggressive behaviour improved with DBS. The findings are similar to the previous published report for cluster headache and help in localization of the proper target in PH for aggressive behaviour.

Disclosures: W.D. Hutchison: None. **R. Micieli:** None. **N. Trujillo:** None. **A. Lopez Rios:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.12/CC9

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Kommission für Klinische Forschung (KKF) of the Klinikum Rechts der Isar der Technischen Universität München grant 8765162

German Federal Ministry of Education and Research (BMBF) grant 01EV0710

Alzheimer Forschung Initiative (AFI) grant 08860 and by the KKF grant 8762754

Elite Network of Bavaria

Chinese Scholar Council (CSC), File No: 2010604026

Title: Dysbalanced intrinsic connectivity of central executive and emotional salience network in borderline personality disorder

Authors: *A. DOLL[1,6], C. SORG[2], C. MENG[1,6], A. WOELLER[3], V. RIEDL[4], A. WOHLSCHLAEGER[5,6];
[1]Neuroradiology, [2]Neuroradiology, Psychiatry, [3]Psychiatry, [4]Neuroradiology, Nuclear Medicine, Neurol., [5]Neuroradiology, Neurol., TUM-NIC Neuroimaging Center, Technische Univ. München, Munich, Germany; [6]Grad. Sch. of Systemic Neurosciences, Ludwig-Maximilians-Universität, München, Germany

Abstract: Borderline personality disorder (BPD) is characterized by "stable instability" of emotions and impulsivity and their regulation. This emotional instability corresponds with a neurocognitive triple network model of psychopathology that suggests that an aberrant relationship between emotional saliency and cognitive control relies on an aberrant integration across intrinsic connectivity networks (ICN) including the salience, default mode, and central executive network (SN, DMN, CEN). The objective of the current study was to analyze whether and how such triple network intrinsic interactions were changed in patients with BPD.
We acquired resting-state functional magnetic resonance imaging (rs-fMRI) data from 16 patients with BPD and 16 matched healthy controls (HC). High-model order independent component analysis (ICA) was used to extract spatiotemporal patterns of ongoing, coherent blood-oxygen-level-dependent signal fluctuations from rs-fMRI data. Inter-ICN time course correlation (inter-network functional connectivity iFC) between ICNs overlapping with canonical SN, DMN, CEN was the main outcome measure of the study.
While patients' iFC of the CEN, critically involved in cognitive control processes, was consistently decreased, only iFC of the SN, associated with emotions, was increased. In particular, a balance index reflecting the relationship of CEN-and SN-iFC across all networks was strongly shifted from CEN to SN connectivity in patients.
Results provide evidence for aberrant triple network iFC in BPD. Our data suggest a shift of iFC

from cognitive control to emotion related brain networks in BPD possibly reflecting the persistent instability of emotion regulation in patients.

Disclosures: A. Doll: None. **C. Sorg:** None. **C. Meng:** None. **A. Woeller:** None. **V. Riedl:** None. **A. Wohlschlaeger:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.13/CC10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: MH36295

MH094356

Title: Association between antidepressant treatment response and EEG alpha: current source density (CSD) spectral measures at rest and time-frequency (TF) measures during a novelty oddball

Authors: *C. E. TENKE, J. KAYSER, J. E. ALVARENGA, K. ABRAHAM, D. M. ALSCHULER, G. E. BRUDER;
Div. Cognitive Neurosci., NYS Psychiatric Inst., New York, NY

Abstract: EEG alpha rhythm has been used as a neurophysiological measure of activation, ranging from relaxation at rest to task-related local cortical synchronization or desynchronization. Although the topographic capacity of high density EEG is limited by volume conduction, CSD transformation provides reference-free measures that represent the neuronal generator pattern underlying alpha. CSD-based frequency principal components analysis (CSD-fPCA) of resting EEG has identified posterior, condition-dependent (eyes open/closed) alpha as a predictor of antidepressant treatment response (Tenke et al 2011). However, alpha also varies during the performance of behavioral tasks, and these changes may be quantified as event-related spectral perturbations (ERSP). The present study combined resting- and task-related (novelty oddball) CSD alpha measures obtained from a 67-channel montage in depressed patients prior to treatment with a serotonergic antidepressant (n = 77) or bupropion (n = 23). Oddball EEG was epoched (-200 to 800 ms) for each condition (target, nontarget, novel), CSD-transformed, and submitted to FFT-based TF analysis to yield ERSP and corresponding baseline spectra. The TF dataset was simplified and quantified by unrestricted PCA (CSD-tfPCA), yielding a prominent, unambiguous factor quantifying alpha desynchronization (ERD), characterized by loadings

peaking at 490 ms (350-700 ms) and 10 Hz (7-12.5 Hz) and scores with a posterior topography for novels and targets, but not nontargets. Resting and task-related baseline EEG alpha were quantified by CSD-fPCA, and submitted to repeated measures ANOVAs with treatment (serotonergic, bupropion), response (responder, nonresponder), and gender as between-factors. The Beck Depression Inventory (BDI) measure of pretreatment depression was used as a covariate because BDI was correlated with condition-dependent alpha at rest ($r = .253$, $p = .01$) and post-stimulus ERD ($r = .212$, $p = .03$). For alpha at rest, we replicated the finding of greater condition-dependent alpha in responders than nonresponders for both treatments. In contrast, task-related alpha (prestimulus baseline or ERD) showed no association with treatment response. However, the overall amplitude of task-related alpha showed a significant treatment by response interaction, where bupropion responders showed less task-related alpha than nonresponders, but this difference was not seen for serotonergic treatment. In conclusion, alpha oscillations at rest and during a novelty oddball task are related to depression and antidepressant treatment response, suggesting distinct processes that modulate default mode activity.

Disclosures: C.E. Tenke: None. **J. Kayser:** None. **J.E. Alvarenga:** None. **K. Abraham:** None. **D.M. Alschuler:** None. **G.E. Bruder:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.14/CC11

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH

Veterans Administration

Title: A transporter-independent site for SSRI action

Authors: *J. SCHAPPI[1], A. CZYSZ[2], M. RASENICK[3];
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Abstract: Chronic treatment of cells or animals with varied antidepressants promotes the redistribution of Galphas from lipid rafts into non-raft membrane fractions. The net result of this redistribution is increased Galphas coupling with transmembrane adenylyl cyclase, and increased cAMP production. These changes are demonstrated via immunoblot, functional assays, and imaging studies. This phenomenon is seen in vivo as well as in cell lines such as C6 glioma and

PC12 pheochromocytoma. These cell lines, however, lack serotonin transporter (SERT), a high-affinity target of selective serotonin reuptake inhibitor-class antidepressants (SSRIs), suggesting that response to monoamine centered antidepressants entails more than inhibition of neurotransmitter reuptake transporters or inhibition of monoamine breakdown. Furthermore, Galphas localization and cAMP production in kidney epithelial cells like COS7 and HEK293 were unchanged by antidepressant treatment. Similarly, membranes from liver and kidney of rats treated chronically with antidepressant did not differ from control animals. One trait shared by cell lines studied, both antidepressant responsive and unresponsive, is a lack of SERT, a binding site of and putative therapeutic target for SSRI antidepressants. Data from this study demonstrate the following: Transgenic expression of SERT in both antidepressant responsive and unresponsive cells does not result in altered antidepressant response compared to wild type. Therapeutically inactive stereoisomer R-citalopram does not cause the characteristic changes in G-protein and cAMP signaling in responsive cell types as seen with S-citalopram. These findings suggest the existence of another yet-unidentified specific target and mediator of SSRI antidepressant action separate from SERT and potential target of new antidepressant drugs.

Disclosures: J. Schappi: None. **A. Czysz:** None. **M. Rasenick:** A. Employment/Salary (full or part-time):; University of Illinois at Chicago. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH, Veterans Administration, Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pax Neuroscience.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.15/CC12

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH, Division of Intramural Research

Title: Pre-treatment subcortical volumes and antidepressant response to ketamine: A predictive analysis

Authors: *C. M. SINCLAIR, A. C. NUGENT, D. A. LUCKENBAUGH, C. A. ZARATE; NIMH / ETPB, NIH, Bethesda, MD

Abstract: Research suggests that dysfunction of the limbic-cortical-striatial-pallidal-thalamic circuit (LCSPT) is connected to the pathophysiology of major depressive disorder (MDD). Previous studies have shown that MDD is associated with subcortical volumetric reductions in the hippocampus (Lorenzetti et al., 2009; Amico et al., 2011), thalamus (Vasic et al., 2008; Kim et al., 2008), caudate (Koolschijn et al., 2009; Kim et al., 2008; Parashos et al., 1998), putamen (Koolschijn et al., 2009; Parashos et al., 1998), and amygdala (Hamilton et al., 2007) as compared to healthy controls. This exploratory study investigated whether the volumes of the thalamus, caudate, putamen, hippocampus, and/or pallidum of patients with MDD correlate with antidepressant response to ketamine, a rapid-acting experimental treatment for depression. MDD subjects (N=31) received a baseline T1 weighted MRI on a 3T scanner, from which all of the above-mentioned subcortical volumes were extracted using FIRST (FMRIB's Integrated Registration and Segmentation Tool, University of Oxford, UK). Brain volumes were then correlated with antidepressant response to ketamine, measured as the percent change in Hamilton Depression scores from baseline to 230 minutes after an intravenous ketamine infusion (0.5 mg/kg over 40 minutes). Pearson correlations showed that smaller bilateral thalamus and caudate volumes correlated with greater antidepressant response (thalamus: $p = .021$; caudate: $p = .020$). Both key areas in the LCSPT network, the thalamus and caudate may serve as important targets for ketamine's antidepressant mechanism of action; this may likely be through increased neuronal spine growth (Duman and Aghajanian, 2012) in these areas susceptible to stress-induced neuronal atrophy. In addition, this relationship between thalamic and caudate volume and antidepressant response to ketamine may serve as a useful biomarker for identifying individuals with a greater predisposition for responding to ketamine, or similar future antidepressant treatment compounds acting on the glutamatergic system.

Disclosures: C.M. Sinclair: None. **A.C. Nugent:** None. **D.A. Luckenbaugh:** None. **C.A. Zarate:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on a patent application for the use of ketamine and its metabolites in MDD. Dr. Zarate has assigned his rights in the patent to the US government but will share a percentage of royalties.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

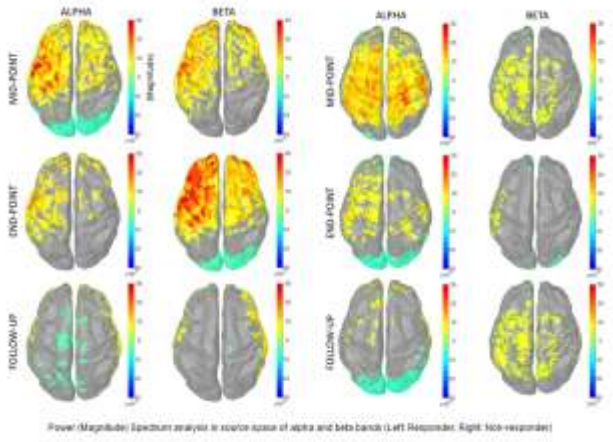
Program#/Poster#: 540.16/CC13

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Differences in beta band activity correlate with clinical response to rTMS for depression

Authors: *Y. PATHAK[1], O. SALAMI[2], S. BAILLET[4], Z. LI[3], C. R. BUTSON[3];
[1]Marquette Univ., Milwaukee, WI; [2]Psychiatry, [3]Neurol., Med. Col. of Wisconsin, Milwaukee, WI; [4]McConnell Brain Imaging Ctr., McGill Univ., Montreal, QC, Canada

Abstract: Major Depressive Disorder (MDD) is characterized as a leading cause of disability worldwide. Current treatment options, including pharmacological interventions, psychotherapy and electroconvulsive therapy, have shown varied efficacy. Therefore, neuromodulation therapies such as deep brain stimulation (DBS), cortical stimulation and repetitive transcranial stimulation (rTMS) are being investigated. Of these, rTMS is attractive due to its focal and non-invasive nature. The goal of this study is to investigate the functional changes that result from rTMS for the treatment of MDD by integrating multimodal imaging. rTMS was administered to the left dorsolateral prefrontal cortex (L-DLPFC) based on a previously published stimulation protocol (fitzgerald,2009). Depressive symptoms were assessed using the Montgomery-Asberg Depression Rating Scale (MADRS). Imaging data (magnetic resonance imaging (MRI), electroencephalogram (EEG), magnetoenceophalogram (MEG), and diffusion tensor imaging (DTI)) were collected before, during and after treatment to evaluate longitudinal changes. Power spectral density (PSD) analysis and functional connectivity analysis were conducted using this data. Results from the MADRS were used to classify subjects as responders or non-responders. PSD results from the MEG data revealed contrasting differences between one responder and one non-responder. For the responder, there was a marked increase bilaterally in the frontal regions for both mid-point and end-point. However, this increase didn't persist at the 2-month follow up. For the non-responder, increase in activity of the frontal regions is lower during treatment and we observed a decrease in power from baseline at follow up, which corresponded to the worsening severity of depressive symptoms. Our results further suggest that functional and structural imaging can be used to evaluate longitudinal changes in functional connectivity and its correlation to clinical response during rTMS treatment for MDD.



Disclosures: Y. Pathak: None. **O. Salami:** None. **S. Baillet:** None. **Z. Li:** None. **C.R. Butson:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.17/CC14

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH grant K23MH079498 (KBW)

NIMH grant RO1MH080193 (CGH)

NIMH grant R01MH059852 (CGH)

International Mental Health Research Organization (OB, CGH, KBW)

Astra-Zeneca (KBW, CGH)

Children's Hospital of Philadelphia (KBW)

Title: Antidepressant treatment decreases glucocorticoid receptor translocation in neuroepithelial cells from living individuals with major depression and controls

Authors: B. R. WILLIS[1,2], *D. SINCLAIR[1], S. JEFFERSON[1], A. MANCEUR[1], O. BERTON[3], C.-G. HAHN[1], K. BORGMANN-WINTER[1,4];
[1]Neuropsychiatric Signaling Program, Univ. of Pennsylvania, Philadelphia, PA; [2]Bryn Mawr Col., Philadelphia, PA; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Background: In major depressive disorder (MDD), elevated stress hormone levels have been consistently reported. Stress hormone dysregulation may be due to impaired function of the glucocorticoid receptor (GR), a key mediator of cellular stress responses and hypothalamic-pituitary-adrenal (HPA) axis negative feedback. Indeed, translocation of GR to the nucleus, an event central to its function, is significantly decreased in vitro, in neuroepithelial cells derived from MDD patients relative to controls. Antidepressants can normalize HPA axis activity in individuals with MDD, but it is not known whether modulation of GR translocation by antidepressants occurs in the brain, and represents a mechanism underlying the therapeutic effects of antidepressants in MDD. Therefore, we employed cultured neuroepithelial cells from the olfactory epithelium (OE), the only cells of putative neural origin which can be propagated from living human subjects, to investigate GR translocation in response to antidepressant treatment in MDD patients and healthy controls. Methods: OE culture neuroepithelial cells from MDD patients (n=3) and controls (n=4) were treated for 2 hrs, 1 day or 4 days with fluoxetine

(an SSRI), desipramine (an SNRI), or duloxetine (a TCA), each at 1uM or 10uM, and GR translocation quantified using automated fluorescent microscopy (ImageXpress, Molecular Devices). Results: Treatment with fluoxetine, desipramine or duloxetine significantly decreased GR translocation in a treatment duration-dependent manner (ANOVA- fluoxetine: $F(2, 48)=3.1$, $p<0.05$; desipramine: $F(2, 48)=5.1$, $p<0.005$); duloxetine ($F(2, 48)=3.4$, $p<0.05$). Greatest decreases in GR translocation were observed after 10uM treatment with fluoxetine for 4 days (-18.5%), desipramine for 4 days (-14.3%) and duloxetine for 1 day (-23.0%), with minimal effects in all conditions after 2 hr treatment. There were no significant differences in antidepressant effects between MDD and control cells, nor were there significant differences between antidepressants. Conclusions: Antidepressant treatment for an extended duration (≥1 day at 10uM) significantly decreases GR translocation in OE-derived neuroepithelial cells of both MDD patients and healthy controls. Future work to investigate the mechanism through which antidepressants act on GR translocation in OE culture cells may identify new mechanisms of antidepressant action on stress hormone disturbances in MDD.

Disclosures: B.R. Willis: None. **D. Sinclair:** None. **S. Jefferson:** None. **A. Manceur:** None. **C. Hahn:** None. **K. Borgmann-Winter:** None. **O. Berton:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.18/DD1

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Successful self-regulation of slow cortical potentials reduces aggression and improves error processing in psychopathic offenders

Authors: *L. KONICAR[1,2], R. VEIT[1], U. STREHL[1], N. BIRBAUMER[1,3];
[1]Inst. of Med. Psychology and Behavioral Neurobio., Eberhard Karls Univ., Tübingen, Germany; [2]Intl. Ctr. for Ethics in the Sci. and Humanities (IZEW), Eberhard Karls Univ., Tuebingen, Germany; [3]Ospedale San Camillo, IRCCS, Venezia-Lido, Italy

Abstract: A history of violence and aggressive behavior is often correlated with a reduced ability to learn and adopt adequate behavior, social rules and norms. Psychopathic offenders show both of these externalizing deficits, together with several affective problems such as a lack of remorse, reduced empathy and deficient anticipatory fear. Prior research has shown, that certain aspects of the externalizing characteristics of psychopathy are related to a reduced cortical activity in self-monitoring and error-processing (Hall et al, 2007; von Borries et al.,

2010).
Therefore we hypothesized that self- regulation of brain activity (Slow Cortical Potential (SCP)-Training as described in Birbaumer et al., 1990) could lead to changes in offenders' aggression as well as to altered error processing.
To examine this question, 14 psychopathic offenders with a severe criminal history performed a letter version of the Eriksen Flanker Task and rated different kinds of aggressive behavior before and after SCP- Training.
Significant reductions in physical and reactive aggression and antisocial tendencies, as well as an improved aggression inhibition were detected after SCP- Training. Furthermore a significant increase in Error Related Negativity and Positivity could be observed, after offenders learned to successful regulate their SCPs.
Our results suggest that self-regulation of cortical activity leads to an improved error processing and aggression regulation in psychopathic offenders.
Supported by Deutsche Forschungsgemeinschaft (DFG) and the International Centre for Ethics in the Sciences and Humanities (IZEW) of the University of Tübingen.
References:
Birbaumer, N., Elbert, T., Canavan, A.G.M. & Rockstroh, B. (1990). Slow potentials of the cerebral cortex and behavior. The American Physiological Society, 70 (1), 1-41.
Hall, J.R., Bernat, E.M. & Patrick, C.J. (2007). Externalizing psychopathology and the error-related negativity. Psychol Sci, 18, 326-33.
von Borries, A.K.L., Brazil, I.A., Bulten, B.H., Buitelaar, J.K., Verkes, R.J. & de Bruijn, E.R.A. (2010). Neural correlates of error-related learning deficits in individuals with psychopathy. Psychological Medicine, 40, 1559-68.

Disclosures: L. Konicar: None. **R. Veit:** None. **U. Strehl:** None. **N. Birbaumer:** None.

Poster

540. **Mood Disorders: Human Biomarkers and Treatment Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 540.19/DD2

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Long-term sertraline treatment increases expression and decreases phosphorylation of glycogen synthase kinase-3b in platelets of patients with late-life major depression

Authors: *H. P. JOAQUIM[1], L. L. TALIB[2], B. S. DINIZ[1], O. V. FORLENZA[2], W. F. GATTAZ[2];
[2]Psychiatry, [1]Psychiatry Intitute HCFMUSP, Lim-27, São Paulo, Brazil

Abstract: Background: Abnormal regulation of glycogen synthase kinase 3-beta (GSK3B) activity has been implicated in the pathophysiology of mood disorders. Many pharmacological agents, including antidepressants, can modulate GSK3B. The long-term treatment with antidepressant may reduce the risk of Alzheimer's disease (AD) in elderly subjects with affective disorders and late-life depression seems to be a risk factor for the development of AD. The neurobiological mechanisms linking these two disorders are unclear, but some proposed mechanisms are: the increased amyloid-beta production during the depressive episode, mediated by the deregulation of the serotoninergic system; the activation of pro-inflammatory cascades, the impairment in the neurotrophic cascades; as well as the abnormal activation of GSK-3B signaling cascade. On the other hand, there is evidence that antidepressants and mood stabilizers, such as lithium, can modulate some of these cascades, in particular GSK3B and neurotrophic cascades.
Objective: Investigate the effect of short- and long-term sertraline treatment on the expression and activity of GSK3B in platelets of patients with late-life major depression.
Methods: 39 no-medicated elderly with major depressive disorder (MDD) were included in this study. The comparison group comprised 18 age-matched, healthy individuals. The expression of total and Ser-9 phosphorylated GSK3B (pGSK3B) was determined in platelets of patients and controls at baseline, and after 3 and 12 months of sertraline treatment by Elisa method.
Results: Depressed patients had higher levels of pGSK3B as compared to controls ($p<0.001$). After 12 months of treatment there was an increase in the expression of total GSK3B ($p=0.05$), in the absence of any significant changes in pGSK3B ($p=0.12$), leading to a reduction in GSK3B ratio ($p=0.001$).
Conclusions: Long-term treatment with sertraline increased GSK3B patient's level to controls values. Our findings suggest that platelet GSK3B ratio may be a useful parameter to establish biochemical changes during antidepressant treatment.

Disclosures: H.P. Joaquim: None. **L.L. Talib:** None. **B.S. Diniz:** None. **O.V. Forlenza:** None. **W.F. Gattaz:** None.**Poster**

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.01/DD3

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: One-time brief vicarious pain is sufficient to enhance fear learning and trigger depression-like behaviors in mice

Authors: *W. ITO, A. MOROZOV;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: (Rationale) Psychological stress is a major trigger of mental disorders, but prevalent rodent models of distress involve physical pain or physical discomfort. Here, we characterized a mouse model of vicarious pain from one-time brief exposure to conspecifics receiving electrical footshocks.
(Experiments and results) Single exposure (duration: 4min) to a distressed cagemate decreased preference to sucrose water measured during the following 12 hours. Moreover, the observers showed enhanced contextual fear and passive avoidance learning, performed 24 hours after the vicarious pain. Despite causing behavioral changes, observing other pain did not increase plasma corticosterone (CORT) more than the control procedure, in which demonstrator did not receive footshocks. A restraint (2hr), however, increased CORT more than the vicarious pain, but did not reduce sucrose preference.
(Discussion) The finding suggests that (1) vicarious pain, even brief, is sufficient to produce lasting changes in mouse brain circuits underlying fear and reward, and (2) the mechanism does not solely involve raise of plasma CORT, as observed in physical stress.

Disclosures: W. Ito: None. **A. Morozov:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.02/DD4

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: PFC-amygdala circuit in a mouse model of vicarious pain

Authors: *A. Y. MOROZOV, W. ITO;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Psychological trauma, even in the absence of physical distress, is the major cause of mental disorders. Yet, most animal models of distress involve physical pain or discomfort. Here we employed a putative mouse model of psychological trauma elicited by vicarious pain and investigated synaptic transmission between the dorso-medial prefrontal cortex (dmPFC) and the basolateral amygdala, the two brain areas implicated in several mental disorders. To selectively study dmPFC-BLA pathway, channelrhodopsin 2 (ChR2) was expressed in dmPFC neurons using a recombinant adeno-associated viral vector. As a traumatic event, the observer animal was exposed to a cage-mate conspecific (the demonstrator) receiving electrical foot-shocks. On the

next day after the exposure, whole cell recording in amygdala slices from the observer mouse were performed to study synaptic responses evoked in BLA principal neurons upon light stimulation of axonal fibers from dmPFC expressing ChR2. Changes were found in both glutamatergic and GABAergic currents evoked in BLA principal neurons. The AMPA/NMDA ratio was reduced, which coincided with an increase in the amplitude of pharmacologically-isolated NMDA receptor-mediated currents evoked by "minimal" stimulation by a narrow beam of light delivered via a small diaphragm. No changes were found in the AMPA receptor mediated currents. The polysynaptic GABAergic currents had faster rise and decay, which indicates that vicarious pain altered the composition and (or) properties of GABAergic neurons recruited by input from dmPFC. Taken together, our findings suggest that vicarious pain alters communication between dmPFC and amygdala by changing both glutamatergic and GABAergic microcircuits involved in interaction between the two structures.

Disclosures: A.Y. Morozov: None. **W. Ito:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.03/DD5

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: MH070727

Title: The role of BDNF-TrkB signaling in dorsal raphe nucleus to mediate antidepressant efficacy

Authors: *M. ADACHI, A. E. AUTRY, L. M. MONTEGGIA;
Univ. Texas Southwestern Med. Ctr., Dallas, TX

Abstract: The molecular basis of antidepressant efficacy for the treatment of major depression is unclear. Several lines of evidence link brain-derived-neurotrophic factor (BDNF) as a target of antidepressant treatment, whereas loss of BDNF has been suggested to underlie certain depressive symptoms. Our laboratory has been investigating the role of BDNF in antidepressant efficacy, as well as depression-like behavior, in mice. We have previously demonstrated that the loss of BDNF in broad forebrain regions attenuates responses to conventional antidepressants in behavioral tests used to predict antidepressant efficacy. More recently, we extended these findings to show that the BDNF-mediated effect on antidepressant efficacy is specific for the dentate gyrus and not other subregions of the hippocampus.

In the present study, we are investigating whether BDNF and its high affinity receptor, TrkB, in dorsal raphe (DR) nucleus contribute to antidepressant efficacy. The DR is a nucleus of serotonergic neurons that provides a large portion of the serotonin innervation to the forebrain, including the dentate gyrus. BDNF and TrkB are highly expressed in the DR and have been suggested to play a role in mediating antidepressant responses but data has not directly examined this possibility. To address this question, we are using a viral mediated gene transfer approach to knockdown BDNF or TrkB selectively in the DR nucleus of adult mice. Adeno-associated virus (AAV) expressing Cre recombinase tagged to GFP (AAV-CreGFP) or AAV-GFP is injected by sterotaxic surgery into the DR of floxed BDNF or floxed TrkB adult mice. The targeted delivery of AAV-CreGFP shows a substantial reduction in Bdnf or TrkB mRNA expression within the DR in comparison to the AAV-GFP injected mice. Mice with localized knockdown of BDNF or TrkB display no obvious abnormalities in overall health and motor function. We are currently assessing emotional behavior as well as responses to conventional antidepressants in these regional specific DR knockdown mice. These data will provide key information on the role of BDNF as well as TrkB in the DR in mediating behavioral responses as well as antidepressant efficacy.

Disclosures: M. Adachi: None. **A.E. Autry:** None. **L.M. Monteggia:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.04/DD6

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R03MH093760

Title: Inhibition of Orexinergic signaling leads to depression-like behaviors in diurnal grass rats

Authors: *S. P. DEATS[1], L. YAN[2];
[1]Psychology, [2]Psychology Dept. & Neurosci. Dept., Michigan State Univ., East Lansing, MI

Abstract: Light has profound effects on humans including mood regulation as exemplified by Seasonal Affective Disorder (SAD) and the beneficial effects of light therapy. However, the underlying neural pathways through which light regulates mood are not well understood. Our previous work has developed the diurnal grass rat, Arvicanthis niloticus, as an animal model of SAD. When housed under a 12:12hr Dim Light:Dark (DimLD) cycle that mimics the lower light intensity of winter, the animals showed increased depression-like behavior compared to the controls housed in bright light during the day (BLD) (Leach et al., 2013). Along with the

behavioral changes, we found that the level of the neuropeptide orexin was reduced in the DimLD animals, suggesting the involvement of orexinergic signaling in light-dependent mood changes. To test this hypothesis further, the present study utilized a selective orexin 1 receptor antagonist (SB-334867). Adult grass rats were kept in the BLD condition for 4 weeks before being exposed to a forced swim test (FST). Based on the immobility time during the FST, paired littermates were assigned into two groups with equivalent average immobility time. On the next day, one group received an injection of SB-334867 (10 mg/kg, i.p.) and the other group received vehicle 4 hrs prior to the FST. The SB-334867 treated group showed significantly longer immobility time compared to the vehicle control group. In addition, a sweet solution preference was reduced in the SB-334867 treated group, but the total amount and daily rhythms of drinking was not affected. The effect of SB-334867 on general locomotor activity and body weight were also assessed, and no significant differences were found between the SB-334867 and vehicle treated groups in those measures. The results suggest that the orexinergic pathway is involved in mood regulation in the diurnal grass rats and that it represents a potential mediator underlying light-dependent changes in mood.
Leach G, Adidharma W, Yan L.Depression-like responses induced by daytime light deficiency in the diurnal grass rat (Arvicanthis niloticus). PLoS One. 8(2):e57115 (2013).

Disclosures: S.P. Deats: None. **L. Yan:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.05/DD7

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R03MH093760

Title: Attenuated orexinergic signaling in a diurnal rodent model of SAD

Authors: *W. ADIDHARMA, L. YAN;
Michigan State Univ., East Lansing, MI

Abstract: Seasonal affective disorder (SAD) is a major depressive disorder recurring in the fall and winter, when there is a reduction of light intensity in our environment. Replenishing the lack of environmental lighting with bright light therapy has been found to be effective in alleviating the depressive symptoms of SAD. Though circadian and monoaminergic systems have been implicated in the etiology of SAD, the underlying neural mechanisms through which light regulates mood are not well understood. To aid in identifying the neural substrates associated

with light-dependent mood changes, we have developed the diurnal grass rat, Arvicanthis niloticus, as an animal model of SAD. By utilizing a 12:12hr Dim Light:Dark (DimLD) paradigm that mimics the lower light intensity in the winter, the animals housed in DimLD showed increased depression-like behaviors compared to animals housed in bright light during the day (BLD) as revealed by forced swim test and sweet solution preference (Leach et al., 2013). Our previous work has found that a selective orexin 1 receptor antagonist (SB-334867) blocks light-induced activation of neurons in the 5-HTergic dorsal raphe (DRN), leading us to suggest the hypothesis that an orexinergic pathway mediates the effects of light on mood (Adidharma et al, 2012). The objective of the present study is to test this hypothesis by investigating the level/intensity of the orexinergic signaling in the grass rats showing depression-like behaviors in DimLD and the controls in BLD. The immunoreactivity of orexin was assessed by immunocytochemistry using standard method. The results revealed a significant reduction in the number of orexin cells in the hypothalamus and in the density of fiber/terminal staining in the DRN of the DimLD group compared to that in the BLD group. The results suggest that he orexinergic pathway plays a role in light-dependent mood fluctuation and in the beneficial effects of light therapy.

Leach G, Adidharma W, Yan L.Depression-like responses induced by daytime light deficiency in the diurnal grass rat (Arvicanthis niloticus). PLoS One. 8(2):e57115 (2013).
Adidharma W, Leach G, Yan L. Orexinergic signaling mediates light-induced neuronal activation in the dorsal raphe nucleus. Neuroscience. 220: 201-7 (2012).

Disclosures: W. Adidharma: None. **L. Yan:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.06/DD8

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: China NNSF 31271092

China CAS KSCX2-EW-Q-18

China NNSF 31171067

China NNSF 61033011

China CAS KSCX2-EW-J-8

Title: Altered pain processing in rats with depressive-like behaviors: A high-resolution EEG/ERP study

Authors: Y.-F. GUO[1,2], Y. XU[1], *J.-Y. WANG[1], F. LUO[1];
[1]Key Lab. of Mental Hlth., Inst. Psychol, Chin Acad Sci., Beijing, China; [2]Grad. Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: Depression and pain disorders are common comorbid conditions and reciprocally affect each other. Significantly reduced acute pain sensitivity has been found in depressed patients and some rodent models of depression. However, mechanisms underlying the impact of depression on pain perception are far from fully understood. This research investigated possible electrophysiological correlates of this disrupted pain experience using the olfactory bulbectomy (OB) model of depression in Sprague-Dawley rats. Electroencephalographic (EEG) activity was continuously recorded through twelve chronically implanted epidural electrodes during the application of brief laser stimuli to the plantar surface of the right hind paw of conscious rats. Behavioral results showed that the likelihood of observing paw withdrawal responses decreased significantly in rats with depressive-like behaviors as compared to controls when the 100 mJ and 140 mJ of laser stimuli were delivered. Time-frequency analysis using wavelet transform and time-domain analysis of EEG signals revealed that the magnitude of laser-induced gamma band oscillations (0-150 ms after stimulus onset, 60-120 Hz) as well as the peak amplitude of evoked potential N70 recorded over the primary somatosensory cortex contralateral to the stimulated side was particularly related to the perceptual variation of identical laser stimuli. Notably, in depressive-like state there was a significant inhibition of this early nociceptive processing subserving the emergence of pain behaviors in rats. In addition, event-related desynchronization of beta rhythm (130-280 ms, 12-30Hz) was markedly disrupted following laser stimulation recorded above the posterior parietal area in OB-treated rats. The present study demonstrates that the modulatory effects of depression on pain perception can be directly observed in the early stages of cortical sensory processing, which may account for the decreased sensitivity to nociceptive stimuli in depressive state. These findings might contribute to a better understanding of the altered responding to the environmental stimuli in depressed individuals.

Disclosures: Y. Guo: None. **Y. Xu:** None. **J. Wang:** None. **F. Luo:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.07/DD9

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: 5P20RR016463

8P20GM103423

Title: Augmented antidepressant properties of acute and chronic administration of imipramine in adult female rats treated prenatally with choline supplementation

Authors: *C. EVANGELISTA, N. K. ZIV, M. J. GLENN;
Psychology, Colby Col., Waterville, ME

Abstract: Emerging data point to the neuroprotective capabilities of prenatal supplementation of the essential nutrient choline. Providing pregnant dams with a choline-rich diet during gestation enhances cognition in offspring (Meck & Williams, 2003). In addition, it attenuates the impact of a host of neural insults, such as neurotoxins (Guo-Ross et al., 2002), seizures (Wong-Goodrich et al., 2010), and brain aging (Glenn et al., 2008). A potential mechanism mediating these behavioral and neural benefits is the enhancement in adult neural plasticity, specifically hippocampal neurogenesis and growth factor content (Glenn et al., 2007; 2008). Hippocampal plasticity is widely recognized as integral in the pathological features of psychological disorders such as depression. Our lab recently demonstrated that developmental choline supplementation exerts antidepressant-like effects in adult female rats (Glenn et al., 2012). This effect was similar in magnitude to the antidepressant imipramine (Porsolt et al., 1978). The goals of the present study were to systematically compare the capacity for prenatal choline supplementation to 1) combat despair in the forced swimming test with acute administration of imipramine and 2) reduce anxiety-like behavior, enhance memory, and increase neurogenesis after chronic administration of imipramine. To do this, timed-pregnant Sprague Dawley rat dams were fed diets containing standard (STD) or supplemental (SUP) levels of choline (AIN76A with 1.1 or 5 g/kg choline chloride, respectively) during embryonic days 11-birth. Offspring were cross-fostered and reared in litters of 10 containing males and females from both diet conditions. Female offspring served as subjects, were weaned at postnatal day (PD) 25, and raised to PD 75 for behavioral testing. For forced swim, rats were placed in an inescapable column of water for 10 minutes and then administered two injections, 1 and 23 hrs later, of saline or 7.5, 15, or 30 mg/kg of imipramine. Despair was assessed 24 hrs later in a 5-min retest in the water column. Despair was attenuated by imipramine in STD rats and its effect was significantly greater in SUP rats, particularly at the 30 mg/kg dose. A month later, rats were chronically exposed to the 7.5 and 15 mg/kg doses, or saline, receiving daily injections for 10 days. Compared to STD rats given saline, STD rats given the higher dose of imipramine and SUP rats given saline showed significantly less anxiety-like behavior. Additionally, SUP rats given imipramine displayed the least anxiety. Presently, memory and hippocampal neurogenesis are under investigation to further uncover interactions between choline supplementation and imipramine.

Disclosures: C. Evangelista: None. **N.K. Ziv:** None. **M.J. Glenn:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.08/DD10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Elucidating novel candidate genes and pathways determining variability in antidepressant response

Authors: *C. LABERMAIER[1], S. H. SCHARF[5], K. V. WAGNER[2], P. WEBER[3], M. UHR[4], M. V. SCHMIDT[2], E. B. BINDER[3], I. SILLABER[6], M. B. MÜLLER[1];
[1]RG Mol. Stressphysiology, [2]RG Neurobio. of Stress, [3]Mol. Genet. of Affective Disorder, [4]Pharmacokinetics and CSF Analysis, Max Planck Inst. of Psychiatry, Munich, Germany; [5]Pharma Res. & Early Development, Discovery Neurosci., F.Hoffmann-La Roche AG, Basel, Switzerland; [6]Phenoquest AG, Munich, Germany

Abstract: Antidepressants are generally effective, but the variability in response and effectiveness is considerably high. Only one-third of patients respond to the first medication prescribed. The identification of genetic factors that might assist in the prediction of an individual's drug response has attracted lot of attention during the last years, but the results from genome-wide association studies for antidepressant response genes have been fairly modest thus far. Moreover, it still remains a mystery why a patient does not respond to the same antidepressant drug in the current depressive episode that was convincingly effective years ago. In this study we were aiming to identify novel candidate genes and molecular pathways, which might be responsible for modulating individual antidepressant response.
Therefore, we designed an animal experimental approach to identify novel candidate genes determining the variability in antidepressant response. We used a genetically homogeneous inbred mouse strain with high innate anxiety (DBA/2J) known to be antidepressant responsive and hypothesized that, when treating a large number of animals chronically with either paroxetine or vehicle, we will be able to identify subgroups of animals which are "good" and "poor" treatment responders according to their performance in the Forced Swim Test. In addition to an overall significant effect of the antidepressant treatment, we detected - despite their genetic homogeneity - a large variability within the paroxetine-treated animals.
Gene expression profiling by means of microarray analysis was then performed with RNA extracted from prefrontal cortex and hippocampus of good and poor treatment responders. We chose these brain regions because brain stimulation and imaging studies in humans have emphasized the role of the prefrontal cortex as a key player in depression and antidepressant response. To enable the identification of genes mediating an early antidepressant response, the same experimental design was applied following 2 weeks of paroxetine treatment corresponding

to the “early response” in the clinical situation. Additional gene expression profiling on RNA extracted from blood samples following 2 weeks of antidepressant treatment might allow the identification of potential peripheral biomarkers associated with early response. Finally, our animal experimental data will be compared and integrated with human genetics data for antidepressant treatment response in patients. We are confident that this translational approach will enable us to identify strong candidates involved in modulation antidepressant response.

Disclosures: C. Labermaier: None. **S.H. Scharf:** A. Employment/Salary (full or part-time):; Full-time Employee at Roche. **K.V. Wagner:** None. **P. Weber:** None. **M. Uhr:** None. **M.V. Schmidt:** None. **E.B. Binder:** None. **I. Sillaber:** A. Employment/Salary (full or part-time):; Full-time Employee at Phenoquest. **M.B. Müller:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.09/DD11

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Center for Neurogenomics and Cognitive Research (CNCR)

Top Institute Pharma project T5-203

Center for Medical Systems Biology (CMSB)

Title: A prison for the mind: Neuronal plasticity in depressive-like states

Authors: *D. RIGA[1], P. VAN BOKHOVEN[1], J. E. VAN DER HARST[3], T. S. HEISTEK[2], P. VAN NIEROP[1], R. C. VAN DER SCHORS[1], J. A. TIMMERMAN[2], A. W. PIENEMAN[1], Y. VAN MOURIK[4], A. N. M. SCHOFFELMEER[4], H. D. MANSVELDER[2], W. J. G. HOOGENDIJK[5], A. B. SMIT[1], S. SPIJKER[1];
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Abstract: Major depressive disorder (MDD) is considered to be the second leading cause of disability world-wide, accounting for more lost productivity than any other psychiatric disorder. This is partially attributed to the cognitive decline that accompanies depression, characterized by persistent impairments related to attention, working and episodic memory and executive functions. The debilitating properties of MDD in the cognitive domain pose questions

concerning the underlying neurobiological mechanisms and efficacy of current therapies.
Wistar rats were subjected to social defeat-induced persistent stress (SDPS) paradigm, in which five defeat encounters are followed by ~3 months of social isolation. During the last 3 weeks of the paradigm, animals were provided with either antidepressant treatment (imipramine) or behavioral therapy (enriched environment). Effects of SDPS and of the two treatment regimes were examined at the behavioral (anhedonia, spatial memory), the electrophysiological (hippocampal long-term potentiation -LTP) and the molecular (hippocampal synaptic proteome) level.
SDPS animals displayed a sustained depressive-like state, including reduced reward anticipation (reflecting anhedonia), diminished hippocampal-dependent spatial recognition memory and reduced maintenance of hippocampus CA1 LTP. Both antidepressant and behavioral therapies were able to rescue the hippocampal LTP deficit and the observed cognitive impairments. Furthermore, the SDPS-induced depressive-like state was characterized by specific changes of the synaptic proteome of the dorsal hippocampus. We are currently assessing whether targeted interventions that reverse these changes can indeed prevent the depression-associated cognitive deficits and reduced plasticity.
Using the SDPS paradigm, an animal model that possesses high face, construct and predictive validity, we can mimic a maintained depressive-like state that models anhedonia and cognitive decline. Both antidepressant treatment and behavioral therapy seem promising on reversing these effects. The identification of the molecular mechanism by which hippocampal cognitive deficits and limited plasticity develop during this state of enduring depression hold great promise for future treatment strategies.

Disclosures: D. Riga: None. **P. Van Bokhoven:** None. **J.E. Van der Harst:** A. Employment/Salary (full or part-time):; Delta Phenomics B.V.. **T.S. Heistek:** None. **P. Van Nierop:** None. **R.C. Van der Schors:** None. **J.A. Timmerman:** None. **A.W. Pieneman:** None. **Y. van Mourik:** None. **A.N.M. Schoffelmeer:** None. **H.D. Mansvelder:** None. **W.J.G. Hoogendijk:** None. **A.B. Smit:** None. **S. Spijker:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.10/DD12

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH R01 MH085069-01

NIH F31 MH095253-01

Title: Sex differences in the effects of intranasal oxytocin administration on social investigation in socially defeated California mice (Peromyscus californicus)

Authors: *M. Q. STEINMAN, C. E. MANNING, S. A. LAREDO, K. I. WALCH, B. C. TRAINOR;
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Abstract: Women are twice as likely as men to be diagnosed with major depression and also respond more poorly to certain antidepressant drug treatments. Oxytocin (OT) is a neuropeptide that regulates mammalian social behavior and stress responses. Three consecutive days of social defeat stress reliably induces social withdrawal in female California mice (Peromyscus californicus), but not males. Interestingly, short term reactivity of OT neurons to defeat stress is similar in both sexes. In contrast, there are sex differences in long term changes in OT systems following defeat stress. Specifically, females but not males exposed to stress exhibit increased OT/c-fos colocalizations in a limbic region, the medioventral bed nucleus of the stria terminalis. Females also show reduced numbers of detectable OT neurons in the paraventricular nucleus of the hypothalamus, a stress responsive region that can release OT into systemic circulation as well as directly within the brain. We administered intranasal OT to males and females (low: 0.8 IU/kg; high: 8 IU/kg; or saline) 2 weeks after social defeat to examine the effects of OT on social interaction. Preliminary results indicate a significant sex*dose interaction on time spent near a novel mouse. Females given the low dose interacted less than females given the high dose or saline. There was a trend for the high dose to increase social interaction levels above saline controls, suggesting a U-shaped dose curve in females. The dose response differed greatly in males with the high dose appearing to decrease social interaction. There were no differences in time spent near an empty cage, suggesting that the effects of OT are specific to social contexts. We also assayed OT plasma levels at several time points. There was no immediate effect of social defeat on plasma OT levels nor were than long term changes in plasma OT levels in females. This suggests that it is unlikely that the increase in c-fos/OT colocalization during defeat reflects increased peripheral release. Furthermore it appears that the long term changes in female oxytocin neural circuits do not correspond with altered peripheral release. These results demonstrate that behavioral effects of oxytocin have different dose dependencies in males and females.

Disclosures: M.Q. Steinman: None. **C.E. Manning:** None. **S.A. Laredo:** None. **K.I. Walch:** None. **B.C. Trainor:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.11/DD13

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: The Danish Advanved Technology Foundation

The Lundbeck Foundation

Title: Prenatal stress induces depressive-like behavior in a sex-specific manner; impact of familiar versus novel environments

Authors: *H. M. SICKMANN[1,2], T. S. ARENTZEN[2], M. P. KRISTENSEN[1], T. B. DYRBY[3], N. PLATH[2];
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Abstract: Stress, including prenatal maternal stress, increases affective disorder morbidity. Furthermore, women appear twice as likely as men to develop stress- and depression-related disorders. Some of the behaviors associated with depression are also found in rat offspring following maternal prenatal stress (PS) incl. increased helplessness and altered anxiety response. Our purpose was to investigate behavioral depression indices following PS and potential differences between male and female offspring.
To this end, pregnant Sprague-Dawley rats were subjected to repeated variable stress on days 13-21 of gestation. The PS paradigm consisted of short-term stressors during the day (e.g. restraint, forced swimming, elevated platform placement) and a long-term stressor during the night (e.g. fasting, lights on). At post natal day 50-70, motor activity, depressive-like (forced swim test), anxiety-like (elevated plus maze, EPM), and sleep behavior (via EEG recordings) was assessed in male and female offspring. In addition, half of PS and control animals, respectively, were exposed to an acute stressor prior to the behavioral tests.
Weight gain during the last part of the pregnancy was significantly reduced in dams exposed to PS. Locomotor activity in a familiar environment (housing cage) during the rodent inactive phase was significantly enhanced in PS females compared to controls (Δ= 49 %, n = 15-37; $p < 0.001$). In contrast, PS decreased locomotor activity in male offspring during the rodent active phase (Δ= 18%, n = 11-33, $p = 0.04$), indicating that PS induces sex-specific behavioral changes. In the EPM, PS per se did not appear to change behavior in either sex. Exposure to an acute stressor, however, increased the amount of time spent in the open arm specifically in control males (Δ= 82%, n = 13-15; $p < 0.001$), and, interestingly, PS appeared to blunt this reaction (Δ= 21%, n = 5-6; $p < 0.36$). In summary, PS induces sex-specific behavioral changes where female offspring appears more vulnerable in a familiar environment whereas male offspring seems vulnerable in a novel environment. The central mechanisms mediating these differences may also contribute to sex-specific sensitivity to stressors and depression propensity in humans.

Disclosures: H.M. Sickmann: None. **M.P. Kristensen:** None. **T.S. Arentzen:** None. **N. Plath:** None. **T.B. Dyrby:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.12/DD14

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: CIHR grant MOP-106562

Title: Regulation of the serotonin autoreceptor (5-HT1A) and transporter (SERT) in the olfactory bulbectomy model of depression and following acute fluoxetine

Authors: M. RIAD[1], S. JOZAGHI[2], L. DESCARRIES[1], *S. M. BOYE[3];
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Abstract: The 5-HT autoreceptor (5-HT1A) and plasmalemmal transporter (SERT) exert tight regulatory control over the activity of 5-HT neurons and appear pertinent to the underlying mechanisms of depression and antidepressant treatment. Previous work in rats has shown that acute treatment with the selective agonist 8-OH-DPAT or the selective 5-HT reuptake inhibitor (SSRI) fluoxetine, leads to internalization of 5-HT1A autoreceptors in 5-HT neurons of the dorsal raphe nucleus (DRN) but not in hippocampus (heteroreceptors). SERT is the primary target of SSRI antidepressants. Considerable evidence suggests that SERT too may be regulated and undergo adaptive changes in response to depression and SSRI treatment. An important shortcoming of these results is that they were obtained in normal rats. The aim of the present study was to investigate changes in the cellular distribution of 5-HT1A and SERT in a murine model of depression, olfactory bulbectomy (OBX). This pre-clinical model produces behavioral, neurochemical and endocrinological consequences that are qualitatively similar to depressive symptomatology. First, we validated our model by measuring locomotor activity in the open field. Two weeks following OBX, lesioned rats displayed the expected hyperresponsiveness to an anxiogenic environment. Animals were then injected with fluoxetine (10 mg/kg, i.p.) or vehicle and sacrificed after one hour. Second, the cellular and subcellular distribution of 5-HT1A and SERT were quantified using electron microscopy after immunogold labeling with specific antibodies against 5-HT1A or SERT, in rats with and without prior fluoxetine treatment and in a group of drug- and lesion-naïve rats. Our results show that in drug-naïve OBX rats, the density of plasmalemmal 5-HT1A autoreceptors is significantly increased in the dendrites of DRN (31% above control) but not in ventral hippocampus (CA3). Acute treatment with fluoxetine abolished

the OBX-induced increase. Quantification of SERT immunolabeling revealed a significant increase in plasmalemmal labeling in both DRN dendrites and hippocampal terminals of OBX rats (24% and 29% above control, respectively), an effect that was not altered by fluoxetine treatment at either site. Thus, the reduction in the plasmalemmal density of 5-HT1A following fluoxetine is consistent with internalization of the autoreceptor, an effect previously observed in normal rats. Further, the increased plasmalemmal density of 5-HT1A and SERT in OBX rats supports the hypothesis of reduced 5-HT function as a contributing factor to depression and further confirms OBX as a valid animal model.

Disclosures: M. Riad: None. **S. Jozaghi:** None. **L. Descarries:** None. **S.M. Boye:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.13/DD15

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH081927

Title: Ablation of serotonergic neurons in the dorsal raphe leads to anhedonia-like behavior in Wistar-Kyoto rats

Authors: *P. C. PUGH, N. L. JACKSON, I. A. KERMAN;
Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: The serotonergic system plays an important role in a host of cognitive and affective functions, including behavioral arousal, homeostasis, sleep-wake regulation, exploration, and different aspects of depressive- and anxiety- like states. The primary source of brain serotonin (5-HT) is from the midline brainstem raphe nuclei, of which the dorsal raphe (DR) contains the greatest number of 5-HT neurons in the brain. Behavioral studies suggest that the DR modulates fear and anxiety-related behaviors; however, the current data are limited to studies that correlate activation of 5-HT neurons with various behaviors. We sought to determine whether elimination of 5-HT neurons in the DR leads to behavioral deficits that are confined to specific aspects of depressive- and anxiety- like states. We made stereotactic injections of the serotonergic toxin 5,7-dihydroxytryptamine (5,7-DHT) directly into the DR of Wistar-Kyoto (WKY) rats, with

control animals receiving an injection of saline of the same volume. After 7 days of recovery, the rats were subjected to a series of behavioral assessments. During the sucrose preference test, lesioned animals displayed lower sucrose preference (61.6 ± 12.5%, N=6) compared to saline-injected animals (92.7 ± 1.1, N=4, p=0.05). Within a social interaction paradigm, the lesioned animals also did not leave a neutral position any faster in the presence of a social partner (13.9 ± 6.9 sec, N=5) than they did for an empty cage (24.57 ± 9.573, N=6, p=0.409), while the sham animals more rapidly approached the social partner (4.9 ± 1.7 sec, N=5) than an empty cage (13.2 ± 2.9 sec, N=4, p=0.037). The animals with DR lesions did not significantly differ from control animals in the open field, elevated plus maze, novelty-suppressed feeding, or forced swim tests. After testing, the animals were sacrificed, and the lesions were confirmed by immunohistochemistry for tryptophan hydroxylase 2 (Tph2), the rate-limiting enzyme for the production of 5-HT in the brain. Our current studies suggest that the lesioning of 5-HT neurons in the DR specifically precipitates anhedonia-like behavior along with deficits in social interaction in the WKY rats. Further studies are underway to expand the cohort of animals and behaviors tested.

Disclosures: P.C. Pugh: None. **N.L. Jackson:** None. **I.A. Kerman:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.14/DD16

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH081927

Title: Effects of maternal separation in behavior and cardiovascular system

Authors: *S. RANA[1], H. NAM[1], N. L. JACKSON[1], P. C. PUGH[1], J. M. WYSS[2], I. A. KERMAN[1];
[1]Dept. of Psychiatry and Behavioral Neurobiol., [2]Cell, Developmental and Integrative Biol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Adverse life experiences during the early developmental period have been shown to have long-term effects on brain, behavior, physiological, and autonomic system in various animal species. Wistar Kyoto (WKY) rats are a proposed model of neuropsychiatric disorders that exhibit depressive and anxiety like behavior with enhanced stress reactivity. This study utilized maternal separation paradigm, a model of adverse early life experience on stress prone WKY rats to investigate the long-term effects on behavior and cardiovascular parameters. The

purpose of the study was to investigate the long term effects of maternal separation (180 min separation), maternal handling (15 min separation), and standard handling (no separation) on the behavior and cardiovascular system. Rat pups were separated from their dams either for 15 (MS15) or 180 (MS180) minutes from postnatal (P) days 1 through 14, whereas another group of rats which were not separated from their dams served as standard handling controls. When the rats reached adulthood (P55), all animals were subjected to a battery of behavioral tests including: novelty-suppressed feeding, open field, elevated plus maze, and social interaction tests to assay anxiety-like behavior. In addition, we utilized forced swim test to measure depressive like behavior. There were only few apparent behavioral differences in the MS15 or MS180 animals in the depressive and anxiety-like behaviors in WKY rats, possibly unsurprising given the baseline behavior of this line. A fraction of maternally-separated rats (MS15 and MS180, N=4 each) were implanted with telemetry pressure probes to obtain chronic cardiovascular measurements over time. Cardiovascular data showed a great level of individual variability in the pressure measurements (systolic, diastolic, and pulse pressures), but the heart rate did not exhibit such variability. No group differences were detected in the pressure parameters analyzed. However, we observed an interesting trend where the circadian periodicity of the mean arterial pressure decreased over time within the MS180 group. In addition, the MS180 rats tended towards an exaggerated tachycardiac response to acute stress (cage change). These preliminary findings suggest that maternally separated rats may exhibit exaggerated stress responses and furthermore might be vulnerable to cardiovascular dysfunction due to aging. We are currently expanding the cohort of animals used to further validate and expand these preliminary findings.

Disclosures: S. Rana: None. **H. Nam:** None. **N.L. Jackson:** None. **P.C. Pugh:** None. **J.M. Wyss:** None. **I.A. Kerman:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.15/DD17

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH081927

Title: Characterization of depressive- and anxiety-like behaviors in different rat strains

Authors: *H. NAM, N. L. JACKSON, P. C. PUGH, S. RANA, S. M. CLINTON, I. A. KERMAN;

Dept. of Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: The Wistar-Kyoto rat (WKY) is a well-established animal model of depression and anxiety. Particularly, the WKY is known for its hyperactive neuroendocrine response to stress. In this study, we characterized WKY's depression- and anxiety-like behavior compared to 3 different strains of male rats (n=12 per strain) - the Spontaneous Hypertensive Rat (SHR), a closely related strain derived from the WKY; the Wistar, the outbred parent strain for both WKY and SHR; and the Sprague-Dawley (SD), an unrelated outbred strain that is widely used in biomedical research. Although behavior characteristics of the WKY rat have already been established, we sought to: 1) obtain further insight into their behavior profiles, including analysis of social interaction behavior and time-dependent changes in forced swimming behavior, not previously described; and 2) directly compare the behavior characteristics of two outbred rat strains that are commonly used as control strains. We replicated previous findings showing high levels of depressive- and anxiety-like behavior and decreased motor drive in WKY animals in the elevated plus maze test (EPM), the open field test (OFT), the novelty-suppressed feeding test (NSF), and the forced swim test (FST). Our data also suggest that the WKY rat has a distinct social interaction behavior profile, showing no preference towards novel male or female partner animals compared to a novel object. While showing similar interest toward the novel object as all the other strains, the WKY rat tended to stay away from the partner rats. In addition, our FST analysis indicates that the WKY rats are more immobile compared to other strains in the beginning of 15-min training phase and become even more immobile in the testing phase on the next day. The WKY rat has previously shown to readily adopt learned helplessness behavior by inescapable footshock paradigm, which is also considered as a depressive-like phenotype in rodents, and here we demonstrated similar behavioral characteristics by forced swimming. Two outbred strains, the Wistar and the SD, possessed similar behavioral characteristics across multiple behavioral tests, with slight differences in overall locomotion and stress-induced defecation. Together these data confirm that the WKY serves as a good animal model for studying depressive- and anxiety-like behaviors. Our future studies will focus on the central mechanisms that shape distinctive behavior profile of the WKY rat.

Disclosures: H. Nam: None. **I.A. Kerman:** None. **S.M. Clinton:** None. **N.L. Jackson:** None. **S. Rana:** None. **P.C. Pugh:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.16/DD18

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Kansas State University

Title: The effects of fluoxetine and differential rearing on the expression of depressive-like states in male rats

Authors: *D. ARNDT, M. CAIN;
Psychology, Kansas State Univ., Manhattan, KS

Abstract: There are many factors that lead to the etiology of depression. Both the environment and serotonin can play a key role in the development of depression. Interestingly, many available antidepressant drugs reduce general locomotor behavior, while they increase escape-directed behavior in the forced swim test (FST), which is indicative of antidepressant-like states (Slattery & Cryan, 2012). The effects of environmental enrichment on FST performance remain unclear, from no effect (Cui et al. 2006) to the production of antidepressant-like states (Brenes et al. 2008; 2009). Likewise, the effects of isolation rearing on depressive-like states are inconsistent, from actually reducing despair (Wongwitdetcha et al. 2006) to increasing it (Heritch et al. 1990) depending upon the duration of the rearing period. Therefore, the current study investigated the effects of differential rearing and fluoxetine administration on locomotor behavior and FST performance. Male Sprague-Dawley rats arrived at the lab at 21 days of age and were randomly assigned to an enriched (EC) or isolated condition (IC) where they differentially reared for 30 days. The effect of fluoxetine (10 mg/kg, i.p.) on locomotor behavior was assessed during a 15-minute locomotor test session. Fluoxetine attenuated locomotor behavior in both EC and IC rats. The effect of fluoxetine on FST performance was then assessed through a 15-minute preswim followed 24 hours later by a 5-minute test session. Rats were subjected to a 15-minute preswim followed by 3 injections of fluoxetine (10 mg/kg, i.p.) or saline 23.5, 5, and 1 hour(s) before the 5-minute test session. All rats had an increase in immobility from the preswim to the test session. Fluoxetine did not, however, decrease immobility in either EC or IC rats compared to saline, suggesting that fluoxetine (10 mg/kg, i.p.) does not produce antidepressant-like effects in enriched or isolated rats. Interestingly, EC-saline and EC-fluoxetine rats exhibited more immobility than their IC counterparts. Additionally, differential rearing did not affect performance during the preswim session, indicating that 30 days of differential rearing does not alter baseline immobility. These results suggest that a 30 day rearing period increases depressive-like behaviors in enriched versus isolated rats in the FST. Therefore, a longer rearing period may be necessary to observe rearing-induced alterations in serotonergic functioning. A longer rearing period may result in both a protective effect of enrichment against depressive behaviors and an EC and IC divergence in the response to fluoxetine.

Disclosures: D. Arndt: None. **M. Cain:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.17/EE1

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIAAA AA015635

NIDA DA019112

Title: Implications of NMDAR GluN2B subunits within the BNST in the antidepressant effects of ketamine

Authors: *K. LOUDERBACK[1,2,3,4], B. D. TURNER[2], T. L. FETTERLY[2], D. G. WINDER[1,3,2,4];
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Abstract: Low-dose ketamine induces rapid and long-lasting antidepressant effects in humans, and this effect has been replicated in rodent models of depression. The mechanisms of this antidepressant effect are not fully understood. Ketamine acts as a noncompetitive N-methyl D-aspartate receptor (NMDAR) antagonist, and studies have demonstrated that the ifenprodil derivative Ro 25-6981 (Ro), which inhibits GluN2B-containing NMDARs, is also capable of reducing depression-like behaviors in rodents. However, recent studies have shown that ifenprodil derivatives have a number of off-target effects at other sites critical for affective disorders, including the norepinephrine and serotonin transporters. Further, systemic administration of compounds such as ketamine and Ro does not allow determination of circuitry crucial for their actions. The GluN2B subunit is highly expressed in the bed nucleus of the stria terminalis (BNST), where it plays an important role in long-term potentiation (LTP). Given multiple studies implicating the BNST in negative affective disorders, we sought to explore the role of the GluN2B subunit within the BNST on affective behavior. First, we demonstrate that systemic ketamine (3mg/kg) and Ro (5mg/kg) decrease latency to feed in the Novelty-Induced Feeding Suppression (NIFS) behavioral paradigm following acute restraint stress. Interestingly, under similar conditions we did not find significant effects of either drug in the elevated zero maze (EZM) or forced swim test (FST). We next utilized a combination of floxed GluN2B mice and stereotaxic delivery of lentiviral Cre-recombinase to delete the grin2b gene specifically from the BNST (BNSTGluN2BKO). Similar to ketamine- and Ro-treated wild type mice, BNSTGluN2BKO mice have significantly reduced latency to feed in the NIFS paradigm but do not show alterations in the EZM or the FST. This effect was specific to GluN2B, as no effect was observed with similar LV-CRE injections into a homozygous floxed glucocorticoid receptor

mouse BNST. To determine the effects of BNST GluN2B deletion or systemic ketamine administration on plasticity within the BNST, we examined field potential induction of LTP in the BNST. We found that in BNSTGluN2BKO mice, an early component of LTP was enhanced compared to control virus (lentiviral GFP) injected mice. We have previously demonstrated that LTP in BNST is disrupted by prior stress. We are currently examining the impact of NMDAR inhibition and deletion on this disruption. In total, these data suggest that GluN2B containing NMDARs in the BNST play an important role in modulation of affective behavior.

Disclosures: K. Louderback: None. **B.D. Turner:** None. **T.L. Fetterly:** None. **D.G. Winder:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.18/EE2

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: FONDECYT 1100322.

Title: Serum microvesicle proteins as potential biomarkers for mayor depressive disorder

Authors: C. GÓMEZ-MOLINA[1], A. LUARTE[1], E. AMPUERO[1], M. SANTIBAÑEZ[1], *U. WYNEKEN[2];
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Abstract: Mayor depressive disorder (MDD) is a multifactorial disease with increasing evidence for the existence of sub-types. As the disease has no clear etiology so far, it is even harder to diagnose subtypes with objective criteria, which in turn will determine the election of appropriate treatments. Animal models of MDD are based on exposure to chronic stress. Previous work in our laboratory demonstrated that two MDD animal models based on movement reduction, either by restriction (RS) in small cages or immobilization (IS) in plastic bags, respond differentially to two antidepressant drugs (ADD), fluoxetine (flx, a serotonin reuptake inhibitor), and reboxetine (rbx, a noradrenaline reuptake inhibitor). We therefore searched for protein markers in the cerebrospinal fluid (CSF) and blood serum to help in the characterization of both models. In the CSF, the metabolic enzyme fructose 1, 6-bisphosphate aldolase C (AldoC), expressed in forebrain astrocytes, was highly increased in the CSF after RS, but not after IS. Based on this, we isolated serum microvesicles and found by western blots that AldoC was present after RS. A second protein differentially present in serum microvesicles of RS animals was Leucin Rich Repeat Neuronal protein 3 (LRRN3), an orphan receptor mainly expressed in brain. Our results

show that serum microvesicles provide a novel and useful tool to identify protein markers present in MDD models, which could in turn help define protein markers for stress induced depressive disorder.

Disclosures: C. Gómez-Molina: None. **A. Luarte:** None. **E. Ampuero:** None. **M. Santibañez:** None. **U. Wyneken:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.19/EE3

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Public Interest Trust Research Aid Fund for Stress-Related Diseases (with Commemoration of Imai kimi)

NIH grant DA032890

William Paterson University

Title: Behavioral roles of cannabinoid type 2 receptor in depression

Authors: *H. ISHIGURO[1,2], K. TABATA[1], N. E. BUCKLEY[3], T. UEMURA[1], N. MOTOHASHI[1], Q.-R. LIU[4], E. S. ONAIVI[2];
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Abstract: Major depression, addiction and other psychiatric diseases are mental health problems associated with stressful events in life with high relapse and recurrence even after treatment. In our previous study, we have demonstrated a high incidence of Q63R polymorphism in the Cannabinoid CB2 Receptor gene (CNR2) in depression, schizophrenia and alcoholics in Japanese population. The receptors are expressed in the brains of mice and rats, and are modulated following exposure to stressors and administration of drugs of abuse. C57BL/6J inbred mice with reduced Cnr2 expression in midbrain appeared to drink more alcohol. Therefore, we further examined a functional relationship of Cb2 receptor in mice, using depression models in this study. C57BL/6J mice subjected to chronic mild stressors for 2 weeks showed higher anxiety in Zero maze test. While Cb2 inverse agonist AM630 increased their anxiety, the agonist JWH015 reduced the anxiety, which was similar to the effect of fluvoxamine. In another study, Poly-IC was injected i.p. to Cnr2 knockout mice, and their

locomotor activity was measured 6, 24, and 48 hours after the injection and compared with those of the wild type controls. Locomotor activity of the mutant mice was relatively maintained in the test cage, in comparison to the activity of the wild type mice that were depressed by Poly-IC. It was interpreted that mice reduced Cb2 function
could not settle themselves down in unfamiliar places following treatment with Poly-IC. The components of the endocannabinoid system may have a role in onset and relapse of stress-related disorders and in some psychiatric diseases. Therefore, CB2 cannabinoid receptors may be a therapeutic target in those psychiatric diseases.

Disclosures: H. Ishiguro: None. **K. Tabata:** None. **N.E. Buckley:** None. **T. Uemura:** None. **N. Motohashi:** None. **Q. Liu:** None. **E.S. Onaivi:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.20/EE4

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Cole Neuroscience Faculty Research Fund/UNH

Title: Ultrasonic vocalizations during intermittent swim stress forecast resilience in subsequent forced swim and spatial learning tests

Authors: *R. C. DRUGAN, T. A. PAPALLO, L. L. CASTRACANE, T. A. WARNER, N. P. STAFFORD;
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Abstract: We previously reported that ultrasonic vocalizations (USVs) during exposure to intermittent swim stress (ISS) predicted protection against stress-induced deficits in an instrumental swim escape test. We now extend these preliminary findings by reporting that rats emitting USVs during ISS also show resistance to stress-induced immobility in the forced swim test (FST) and spatial learning deficits in the Morris water maze (MWM). Rats were exposed to either 80-5 sec exposures to cold water swims (average of one per min, ISS) or run through the procedure in the absence of water (confined control, CC). We recorded the number and duration of ultrasonic vocalizations by a high frequency microphone connected to a computerized data capture system (Labview). Experiment #1: twenty-four hours later separate groups of both ISS and CC rats were exposed to a 5 min forced swim test and behaviors (e.g., swimming, climbing, immobility) were scored during FST. For Experiment #2, twenty-four hours post ISS, spatial learning was assessed in the MWM (18 massed trials). In both experiments, only a small number

of rats emitted USVs during the ISS. However, every one that vocalized showed reduced immobility in the FST and proficient spatial learning in the MWM. These findings provide further evidence that this non-invasive measure serves as a predictor of future resilience, may uncover previously unappreciated neural systems associated with resilience, and hasten novel drug discovery and more effective pharmacotherapy for depression.

Disclosures: R.C. Drugan: None. **T.A. Papallo:** None. **L.L. Castracane:** None. **T.A. Warner:** None. **N.P. Stafford:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.21/EE5

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH082933

Title: Mechanisms underlying the effects of vagal nerve stimulation

Authors: *A. P. SHAH, Y. CHUNG, F. R. CARRENO, A. FRAZER;
Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, SAN ANTONIO, TX

Abstract: Vagal nerve stimulation (VNS) is an FDA-approved therapy for treatment resistant depression (TRD). Whereas there is an anatomical rationale for the antidepressant-like effects of VNS, the molecular mechanisms underlying these effects have not been characterized in depth. We have shown that similar to classical antidepressant drugs, acute or chronic administration of VNS causes activation of TrkB, the receptor for BDNF and NT-4/5. However, the role of this receptor in the behavioral effects of VNS remained to be investigated. We have addressed this question using K252a, a non specific tyrosine kinase inhibitor that targets TrkB and have used the Novelty Suppressed Feeding Test (NSFT) as the behavioral output. We have previously shown that chronic VNS (10 days), like desipramine (DMI), leads to a reduction in latency to eat familiar food in a novel box which is indicative of an anxiolytic-like effect of the treatment. We now find that this effect of chronic VNS is blocked after repeated intracerebroventricular injections of K252a. Interestingly, neither the anxiolytic-like effect (in the NSFT) nor the antidepressant-like effect (in the Forced Swim Test) of DMI was blocked by K252a. Food consumption within the home cage and locomotor activity were monitored in order to control for confounding effects of the drugs/VNS. These observations highlight a difference in the mechanisms of action of VNS and antidepressant drugs.
In order to explore the broader effects of VNS and compare these with effects of antidepressant

drugs, we have carried out whole genome microarray studies using hippocampal tissue from animals that have been chronically treated with VNS or DMI. Preliminary analysis reveals changes ($p<0.05$ and fold change>1.5) in expression of potentially relevant genes in response to VNS. For instance, expression of the gene, *Ttr* is significantly upregulated 16.7-fold ($p=1.24x10^{-7}$). *Ttr* codes for transthyretin, which binds to and transports thyroxine and retinol. Clinical studies have shown that depressed patients have low CSF levels of transthyretin.

Disclosures: A.P. Shah: None. **Y. Chung:** None. **F.R. Carreno:** None. **A. Frazer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cyberonics Inc.. F. Consulting Fees (e.g., advisory boards); Lundbeck, Eli Lilly & Co., Takeda Pharmaceuticals International, Inc, Cyberonics Inc..

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.22/EE6

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Effects of SSRI and SNRI antidepressant drugs on schedule-induced polydipsia in rats: Evaluation of α2 adrenoceptor antagonism with yohimbine

Authors: S. M. MOONEY-LEBER[1], M. D. BERQUIST, II[1], A. L. PEHRSON[2], N. P. PORTER[3], J. H. PORTER[3], *A. PRUS[1];
[1]Psychology, Northern Michigan Univ., MARQUETTE, MI; [2]H. Lundbeck A/S, Paramus, NJ; [3]Psychology, Virginia Commonwealth Univ., Richmond, VA

Abstract: Antidepressant drugs require several weeks to exhibit efficacy for the treatment of depression or anxiety disorders. This delayed onset appears to be modeled in the schedule-induced polydipsia paradigm in rodents, in which several days of antidepressant administration may be necessary to significantly reduce water consumption. The present study sought to replicate an inhibition in scheduled-induced water consumption in rats observed after several days of the selective serotonin reuptake inhibitor (SSRI) fluoxetine administration as reported in earlier studies, as well as to extend these previous findings by evaluating the serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine. Further, given that α2 adrenoceptor desensitization may contribute to the antidepressant effects of duloxetine, the present study also sought to determine if administration of the alpha2 adrenoceptor antagonist yohimbine could potentiate fluoxetine's effects on schedule induced polydipsia. In Experiment 1 a 10.0 mg/kg dose (p.o.) of fluoxetine required 6 days of repeated administration to significantly reduce water

consumption; whereas, a 30.0 mg/kg dose significantly reduced water consumption on day 1. A 30.0 and 100.0 mg/kg dose of duloxetine (p.o.) decreased water consumption after 3 days and 1 day, respectively. In Experiment 2 a dose of 4.0 mg/kg (i.p.) fluoxetine failed to significantly reduce water consumption over 6 days of injections; whereas, an 8.0 mg/kg dose reduced water consumption on day 3. Co-administration of the sub-effective 4.0 mg/kg dose of the SSRI fluoxetine with sub-effective doses of yohimbine (1.25 and 2.5 mg/kg) led to a significant decrease in water consumption on the second day of administration with 1.25 mg/kg yohimbine and on the first day of administration with 2.5 mg/kg yohimbine. The present results confirm previous findings that inhibition of serotonin reuptake alone is sufficient to reduce water consumption in the schedule-induced polydipsia paradigm. In addition, the results for the α2 antagonist yohimbine (in combination with fluoxetine) support the suggestion that blockade of α2 adrenoceptors may significantly reduce the response time for antidepressant efficacy in humans.

Disclosures: S.M. Mooney-Leber: None. **M.D. Berquist:** None. **A.L. Pehrson:** None. **N.P. Porter:** None. **J.H. Porter:** None. **A. Prus:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.23/EE7

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Identification of a stress-vulnerable, treatment-resistant, ketamine-sensitive genetic line in the chick anxiety-depression model

Authors: *S. W. WHITE[1], K. J. SUFKA[1,2,3];
[1]Dept. of Psychology, Univ. of Mississippi, University, MS; [2]Res. Inst. of Pharmaceut. Sci., [3]Dept. of Pharmacol., Univ. of Mississippi, Univeristy, MS

Abstract: Current antidepressant pharmacotherapies lack effectiveness in significant portions of patients suffering from major depression. Many in this clinical population show stress-vulnerability and treatment-resistance (failure to respond to two classes of FDA approved antidepressants), but do respond to the *N*-Methyl-D-aspartate (NMDA) antagonist ketamine. The introduction of novel therapies for treatment-resistant depression is hindered by translational challenges with existing preclinical screening models. Recent research in the chick anxiety-depression model identified two strains in which one displays stress vulnerability (Black Australorp) and the second stress resilience (Production Red) as measured by onset of behavioral

despair. This current study sought to explore whether these two lines display differential sensitivities to the tricyclic antidepressant (TCA) imipramine, the selective norepinephrine reuptake inhibitor (SNRI) maprotiline, the selective serotonin reuptake inhibitor (SSRI) fluoxetine, and ketamine. Socially-raised chicks (Ideal Poultry, Cameron, TX) were tested in isolation (90 m) in separate sound-attenuating chambers at 5-6 d post-hatch 15 m after receiving IP injections of vehicle or one of several doses of drug probes. Distress vocalization served as the dependent measure. Here, we replicate the Black Australorp strain is vulnerable to isolation stress. Further, this strain is also treatment resistant to imipramine and fluoxetine but shows antidepressant sensitivity to ketamine. Our findings suggest that the chick anxiety-depression model using the Black Australorp line may prove useful in pre-clinical screening of novel antidepressant targets for use in treatment-resistant depression.

Disclosures: S.W. White: None. **K.J. Sufka:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.24/EE8

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH/NIGMS (2 SO6 GM08016-39)

NIH-RCMI 2 G12 RR003048

HU College of Medicine Bridge Grant

Title: Antidepressant effects of resveratrol in an animal model of depression

Authors: *L. L. HURLEY, L. AKINFIRESOYE, O. KALEJAIYE, Y. TIZABI;
Pharmacol., Howard Univ., WASHINGTON, DC

Abstract: Resveratrol (3, 4', 5-trihydroxy-trans-stilbene) is a natural non-flavonoid polyphenol antioxidant extracted from red grapes in the processing of wine. Initially it was studied for its potential as anticancer drug, and later was recognized for its ability to reduce cardiovascular disease. More recently resveratrol was shown to alleviate depressive-like symptoms in mice, postulated to be due to its induction of central biogenic amines and a decrease in neuro-inflammatory markers. The purpose of this study was to investigate whether resveratrol would manifest an antidepressant effect in Wistar-Kyoto rats, a putative and non-induced animal model of depression, which are resistant to conventional selective serotonin reuptake inhibitors (SSRIs).

Additionally, since hippocampal brain-derived neurotrophic factor (BDNF) has been implicated in antidepressant effects of many drugs, we evaluated the level of this protein in both the hippocampus and frontal cortex. Adult male WKY rats were subjected to two doses of resveratrol (10 and 40 mg/kg, i.p.) and their behavior in the open field locomotor activity (OFLA), forced swim test (FST: a measure of helplessness), and sucrose preference test (SPT: a measure of anhedonia) was evaluated after a single acute injection (20 min) or 18-20 h following the last of 7 daily injections. Both acute and chronic treatment with resveratrol resulted in a dose-dependent decrease in FST, and chronic treatment with the higher dose increased sucrose consumption. OFLA was not affected by any dose of resveratrol. Parallel to the observed behavioral effects, the level of hippocampal BDNF, but not frontal cortex, was also dose-dependently elevated after chronic resveratrol administration. These findings support an antidepressant-like effect of resveratrol, possibly mediated through hippocampal BDNF. Thus, resveratrol may have therapeutic potential, particularly in at least the subpopulation of treatment resistant depressed patients.
Supported by: *NIH/NIGMS (2 SO6 GM08016-39), NIH-RCMI 2 G12 RR003048 and HU College of Medicine Bridge Grant*

Disclosures: L.L. Hurley: None. **L. Akinfiresoye:** None. **O. Kalejaiye:** None. **Y. Tizabi:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.25/EE9

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH/NIGMS (2 SO6 GM08016-39)

NIAAA (P20 AA014643)

NIH-RCMI 2 G12 RR003048

HU College of Medicine Bridge Grant

Title: Nicoitne blocks alcohol-induced decreases in hippocampal BDNF and synapsin: Implication for smoking-drinking co-morbidity

Authors: *O. O. KALEJAIYE, R. E. TAYLOR, Y. TIZABI;
Pharmacol., Howard Univ. Col. of Med., Washington, DC

Abstract: We have reported that nicotine attenuates alcohol-induced depression in Wistar rats and hence the opposing effects of these two substances on mood may be a contributory factor to their co-abuse (Kalejaye et al SFN abst 2012). Although the exact neurobiological substrates of depression in general, and alcohol-induced depression in particular, are not known, it has been suggested that the action of brain derived neurotrophic factor in different brain regions and specifically in hippocampus may be crucial in mood regulation and effectiveness of antidepressants. In addition, changes in synaptogenesis have been implicated in mechanism of fast acting antidepressants. Here, we sought to determine whether alcohol-induced depression is associated with a reduction in hippocampal BDNF and/or synaptogenesis, and whether nicotine may attenuate these neurochemical effects of alcohol. Adult male Wistar rats were injected (i.p.) with alcohol (1.0 g/kg), nicotine (0.3 mg/kg) or their combination once daily for 21 days. Controls received saline. These injections were identical to the previously evaluated behavioral effects where the helplessness and anhedonia induced by alcohol were shown to be attenuated by nicotine. In this case, however the rats were sacrificed 24 after the last injection for measurement of hippocampal BDNF and synapsin, a marker of synaptogenesis. These proteins were evaluated by Western blot. Parallel to the behavioral changes chronic alcohol resulted in significant decreases in hippocampal BDNF and synapsin. Nicotine by itself did not affect the levels of these markers, but completely blocked the effects of alcohol. Thus, hippocampal changes in neurogenesis and synaptogenis may underlie alcohol-induced depression and their normalization by nicotine may underlie the effectiveness of nicotine as an antidepressant.
Supported by: NIH/NIGMS (2 SO6 GM08016-39), NIAAA (P20 AA014643), NIH-RCMI 2 G12 RR003048 and HU College of Medicine Bridge Grant

Disclosures: O.O. Kalejaiye: None. **R.E. Taylor:** None. **Y. Tizabi:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.26/EE10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant 1R21MH099562

Title: Sex-specific regulation of the microRNA transcriptome by stress

Authors: *M. L. PFAU, G. E. HODES, J. FENG, S. A. GOLDEN, H. M. CATES, D. J. CHRISTOFFEL, M. HESHMATI, H. ALEYASIN, L. SHEN, S. J. RUSSO;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Adult women are twice as likely as men to develop major depression, although the precise molecular mechanisms underlying sexual dimorphism in depression susceptibility are unknown. We have developed a stress paradigm—subchronic variable stress (SCVS)—to model these sex differences in mice. Female mice subjected to SCVS exhibit depression-like behavior after 6 days of stress exposure, whereas male mice exhibit depression-like behavior only after 28 days of variable stress. Thus, this model has validity with relation to the increased prevalence of stress disorders in women. To identify the potential mechanisms driving these behavioral differences, we have profiled sex differences in gene expression in the mouse nucleus accumbens (NAc) following SVCS using next generation mRNA sequencing (RNA-Seq). We found that males exhibit a robust transcriptional response to 6-day SCVS. However, less than 3% of the stress-regulated genes identified by RNA-Seq were similar between the sexes. To examine potential mechanisms regulating this sex difference in transcriptional response to stress, we have performed next generation small RNA-Seq on NAc tissue from intact male and female mice subjected to SCVS. Here, we report significant sex differences in microRNA transcriptional profiles that correlate with behavioral susceptibility or resilience to stress and mRNA transcription patterns.

Disclosures: M.L. Pfau: None. **G.E. Hodes:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Johnson & Johnson. **J. Feng:** None. **S.A. Golden:** None. **H.M. Cates:** None. **D.J. Christoffel:** None. **M. Heshmati:** None. **H. Aleyasin:** None. **L. Shen:** None. **S.J. Russo:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Johnson & Johnson.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.27/EE11

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: VISN19 MIRECC

University of Utah USTAR

Title: Hypobaric hypoxia induces depression-like behavior in female but not male Sprague Dawley rats

Authors: *S. KANEKAR[1,2], P. OLSON[1], O. BOGDANOVA[1], K. D'ANCI[3,4], P. RENSHAW[1,2]; [1]Brain Inst., [2]Dept. of Psychiatry, Univ. of Utah, Salt Lake City, UT; [3]Dept. of Psychology, Tufts Univ., Medford, MA; [4]Dept. of Psychology, Salem State Univ., Salem, MA

Abstract: Demographic studies show that higher rates of depression and suicide, the most adverse impact of unresolved depression, correlate with altitude of residence. Selective serotonin reuptake inhibitors (SSRIs), the most commonly prescribed antidepressants, function by increasing synaptic exposure to serotonin. However, living at altitude exposes people to hypobaric hypoxia, which lowers rat brain serotonin levels, and SSRIs show low efficacy in mice with low brain serotonin. Hypobaric hypoxia may thus reduce SSRI efficacy, likely causing higher rates of SSRI-resistant depression at altitude. We have established an animal model for altitude-related depression to study SSRI efficacy at altitude. We first determined whether hypobaric hypoxia can induce depression-like behavior in rats. Sprague Dawley rats were housed at altitude simulations of 20,000 ft (20K), 10,000 ft (10K) or sea level (SL) using altitude chambers, or at local conditions of 4,500 ft (4.5K, altitude of Salt Lake City, UT) for 1 week. Rats were then tested in the forced swim test (FST), an established behavioral test used to identify depression-like behavior and screen antidepressants in rats. Behavior in the FST was scored as time spent swimming, climbing or immobile, and latency to immobility was measured. Increased immobility and reduced latency to immobility in the FST are viewed as signs of depression-like behavior. Results: Female rats exhibit incrementally more depression-like behavior with exposure to altitude for a week, while males do not. Females housed at SL (n=10) spent 50% of their time in the FST in active, escape-seeking behavior, which dropped to 33% in the 4.5K group (n=24), and to 21% in 10K (n=9) or 20K (n=11) groups. Female rats at altitude exhibit incrementally more immobility ($F(3, 50)=5.2$, $p=0.007$) and lower latency to immobility ($F(3, 50)=10.9$, $p<0.0001$) (p values corrected with Bonferroni post-hoc test). Females at altitude swam less than those at SL ($F(3, 50)=11.7$, $p<0.0001$) while climbing was unchanged, implying that hypobaric hypoxia impacts brain serotonin but not dopamine levels. In contrast, male rats in the SL (n=11), 4.5K (n=16) or 20K (n=10) groups exhibit identical FST behavior: 23-25% of time spent active and 75-77% immobile, with a similar latency to immobility across groups. Gender differences in depression, antidepressant response, and serotonin turnover are seen in both animal (Dalla et al., 2009) and clinical studies (Keers et al., 2010), implying the potential use of a gender-based approach to antidepressant therapy. Analysis of brain monoamines in the 4 altitude groups and SSRI efficacy tests at altitude are in progress. Funded by USTAR and VISN19 MIRECC.

Disclosures: S. Kanekar: None. **P. Olson:** None. **O. Bogdanova:** None. **K. D'Anci:** None. **P. Renshaw:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.28/EE12

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Dissociable effects of the noncompetitive NMDA antagonists ketamine, phencyclidine (PCP), MK-801, and other glutamatergic ligands in the differential-reinforcement-of-low-rate (DRL) 72 sec task

Authors: *B. L. JOSEPH, T. M. HILLHOUSE, F. F. STEELE, J. H. PORTER;
Psychology, Virginia Commonwealth Univ., Richmond, VA

Abstract: Major depressive disorder (MDD) is the most common mood disorder in the United States with approximately 36% of MDD patients categorized as being treatment resistant (i.e. no/minimal symptom reduction after adequate treatment duration). Clinical research has demonstrated that ketamine produces rapid and prolonged antidepressant effects in treatment-resistant patients. Preclinical research is currently evaluating the possible underlying mechanism(s) responsible for these robust antidepressant effects. Our laboratory has previously shown that the noncompetitive N-Methyl-D-aspartate (NMDA) antagonist ketamine produces antidepressant-like effects in the differential-reinforcement-of-low-rate (DRL) 72 sec operant task, which has been used to selectively screen antidepressant drugs since the 1980's. A ligand demonstrates antidepressant-like effects in this preclinical assay if the number of reinforcers earned are increased, responses emitted are decreased, and produces a rightward shift in the interresponse times (IRT) distribution. The aim of the present study was to determine if the antidepressant-like effects of ketamine in the DRL 72 sec task are unique to ketamine or shared by other noncompetitive NMDA antagonist (i.e. phencyclidine (PCP) and MK-801), as well as other glutamatergic ligands. Ketamine (10.0 mg/kg) produced an antidepressant-like effect in the DRL-72 sec task by increasing the number of reinforcers earned, decreasing the number of responses emitted, and producing a rightward shift in the IRT distribution. The high dose of PCP (10.0 mg/kg) disrupted behavioral responding and did not demonstrate an antidepressant profile in the DRL 72 sec task. Conversely, MK-801 (0.05 and 0.1) produced a psychostimulant-like effect by decreasing the number of reinforcers earned, increasing the number of responses emitted, and producing a leftward shift in the IRT distribution. The remaining glutamatergic ligands tested appear to have mixed results. These data indicate that ketamine, PCP, and MK-801 produced dissociable effects in the DRL-72 sec task and further suggest that ketamine may have greater antidepressant efficacy. Additionally, these results suggest that the underlying mechanism responsible for the antidepressant effects of ketamine may be unique to ketamine and not to all NMDA antagonists.

Disclosures: B.L. Joseph: None. **T.M. Hillhouse:** None. **F.F. Steele:** None. **J.H. Porter:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.29/EE13

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: The noncompetitive NMDA antagonist ketamine, but not MK-801, produces antidepressant-like effects in rats responding on a differential-reinforcement-of-low-rate (DRL) 72 second operant schedule

Authors: *J. H. PORTER, T. M. HILLHOUSE;
Psychology, Virginia Commonwealth Univ., Richmond, VA

Abstract: Major depressive disorder (MDD) is the most common mood disorder in the United States with a lifetime and 12-month prevalence of 16.6% and 7.1%, respectively. Current antidepressant treatments primarily target the monoamine neurotransmitters (i.e. serotonin, norepinephrine, and dopamine) in an attempt to enhance the presence of monoamine neurotransmitters in the synaptic space. Unfortunately, approximately 36% of patients are treatment resistant (i.e. they fail to response to 2 or more antidepressant treatments). The noncompetitive N-Methyl-D-aspartate (NMDA) antagonist ketamine has been shown to produce rapid and prolonged antidepressant effects in treatment-resistant MDD patients following both single and repeated administration of a low, subanesthetic dose. The current study evaluated the antidepressant effects of ketamine and the more potent and selective noncompetitive NMDA antagonist MK-801 (dizocilpine) using the differential-reinforcement-of-low-rate (DRL) 72 sec operant task in rats. Ligands demonstrate antidepressant-like effects in this preclinical assay if the number of reinforcers earned are increased, responses emitted are decreased, and produces a rightward shift in the interresponse times (IRT) distribution. The noncompetitive NMDA antagonist ketamine (10.0 mg/kg) and the agonist NMDA (30.0 mg/kg) significantly increased reinforcers, decreased responding, and produced a rightward shift in the IRT distribution. MK-801 produced psychostimulant-like effects by decreasing reinforcers, increasing responses, and producing a leftward shift in the IRT distribution. The tricyclic antidepressant imipramine and the selective serotonin reuptake inhibitor fluoxetine both produced antidepressant-like effects; whereas, the dopamine agonist d-amphetamine produced psychostimulant-like effects similar to the effects produced by MK-801. Combination testing revealed that a sub-effective dose of ketamine (3.0 mg/kg) antagonized the antidepressant-like effects of NMDA (30.0 mg/kg). However, a sub-effective dose of NMDA (10.0 mg/kg) had no effect on the antidepressant-like effects of ketamine (10.0 mg/kg). While it may seem counterintuitive for both an antagonist and

agonist at the same receptor to produce similar effects, ketamine and NMDA have different binding sites at the NMDA receptor and may work through different mechanisms. Ketamine and MK-801 produced dissociable effects in the DRL-72 sec task and this suggests that the antidepressant effects of ketamine may be mediated by pharmacological mechanism(s) exclusive to ketamine, but not other NMDA antagonists.

Disclosures: J.H. Porter: None. **T.M. Hillhouse:** None.

Poster

541. **Mood Disorders: Animal Models II**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 541.30/EE14

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: The Scientific and Tecnological Research Council of Turkey, National Young Researchers Career Development Program

Title: The role of FGF2-AS in stress and depression-like behavior in rats

Authors: *E. EREN KOCAK[1,2], K. BASAR[2], M. YILMAZ[1], Y. AYHAN[2], T. DALKARA[1,3]; [1]Inst. of Neurolog. Sci. and Psychiatry, [2]Dept. of Psychiatry, Fac. of Med., [3]Dept. of Neurology, Fac. of Med., Hacettepe Univ., Ankara, Turkey

Abstract: There is a growing body of evidence on the association of FGF2 with depression. For example, chronic stress decreases and antidepressant administration increases FGF2 expression in the prefrontal cortex and hippocampus. Moreover systemic and intracerebroventricular (icv) administration of FGF2 has been shown to have antidepressant effects. Unlike most mammalian genes, antisense (AS) strand of the FGF2 gene is also transcribed into an RNA (FGF2-AS) that is thought to regulate the expression of FGF2. We hypothesized that chronic stress would increase FGF2-AS levels and overexpression of FGF2-AS would have depressogenic effects. First we investigated the effects of chronic restraint stress on the expression of FGF2 and FGF2-AS in male Sprague Dawley rats at three time points: 2h, 6h and 24h after the last stress. Interestingly we found a decrease in the protein expression of both FGF2 and FGF2-AS in the prefrontal cortex following chronic stress exposure. Then we investigated the effects of FGF2-AS overexpression by repeated icv injections of an expression vector bearing FGF2-AS. We found that FGF2-AS overexpression decreases immobility time in forced swim test (FST). Our findings indicate that FGF2-AS is important for the regulation of mood. Contrary to our hypothesis, chronic increase in the expression of FGF2-AS has antidepressant effects on FST, similar to the effects of FGF2.

Disclosures: E. Eren Kocak: None. **K. Basar:** None. **M. Yilmaz:** None. **Y. Ayhan:** None. **T. Dalkara:** None.**Poster**

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.01/EE15

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: An animal model of recurrent depression: sensitized depression-like behavior when rats are re-exposed to chronic mild stress

Authors: *J. L. REMUS, D. JAMISON, J. D. JOHNSON;
Biol. Sci., Kent State Univ., Kent, OH

Abstract: Major depression is a debilitating disorder, characterized by feelings of worthlessness, persistent sadness and lack of motivation. Depression is also recognized to be a chronic, recurrent disorder, and each depressive episode increases an individual's susceptibility to future occurrences. The current study aimed to develop an animal model of recurrent depression in order to examine possible biological mechanisms responsible for this increased susceptibility. We hypothesized that animals with a prior depressive episode would be sensitive to future stressors, causing the animals to display depressive-like behaviors more rapidly or to a greater extent. Fisher rats (n=15) were exposed to chronic mild stress for 35 days or remained in their home cage as controls. During the initial stress, animals showed a slow decrease in sucrose consumption. Following the 35 days, animals went through a 20 day recovery phase where no stressors were present and animal showed a steady increase in sucrose consumption back to baseline levels. At this time animals were re-exposed to the chronic mild stress for 15 days. A rapid decline in sucrose consumption was observed. Linear regression lines were calculated for each animal's sucrose consumption during the first and second stressor exposure and a paired t-test of the slope of each line revealed re-exposure to stress resulted in a significantly more rapid decline in sucrose consumption compared to that observed during the initial stressor exposure.

Disclosures: J.L. Remus: None. **D. Jamison:** None. **J.D. Johnson:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.02/EE16

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: VA Grant RX000150

Title: Phospholipase D - mTOR signaling is compromised in a rat model of depression

Authors: *P. FENG, Y. HU, C. HUANG;
Res., Case Western Reserve Univ/Cleveland VA, CLEVELAND, OH

Abstract: Depression is associated with structural and neurochemical changes in limbic structures, including the hippocampus, that control emotion and mood. Structural abnormalities such as decrease in hippocampal cell proliferation, neurogenesis and hippocampal volume, and loss of neurons and glial cells have been widely reported in physical and psychosocial stress paradigms and animal model of depression, but corresponding neurochemical changes are largely unknown. Using neonatal clomipramine (CL)-treated rats as a model to elucidate the association of phospholipase D (PLD) and mammalian target of rapamycin (mTOR) signaling with depressive pathology, we found that the hippocampus of CL-treated rats showed significantly down-regulation of PLD1 expression and attenuation of PLD activity which leads to the less formation of phosphatidic acid (PA), an activator of mTOR, and free choline, a potential biomarker for depression. With lower PA levels which could affect mTOR signaling, we further observed that the phosphorylation of p70S6 kinase, one of the downstream effecors of mTOR, was also significantly decreased in the hippocampus of CL-treated rats compared to the controls. Down-regulation of PLD1 expression, PLD activity and p70S6 phosphorylation was also found in the hypothalamus and frontal cortex with CL-treated rats. Our results indicate that PLD-mTOR signaling is associated with depressive disorder.

Disclosures: P. Feng: None. **Y. Hu:** None. **C. Huang:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.03/EE17

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Tricyclic antidepressant amitriptyline indirectly increases the proliferation of adult dentate gyrus-derived neural precursor cells via inducing FGF2 secretion from astrocytes

Authors: *S. BOKU[1], K. HISAOKA-NAKASHIMA[2], S. NAKAGAWA[3], A. KATO[3], N. KAJITANI[2], T. INOUE[3], I. KUSUMI[3], M. TAKEBAYASHI[4];
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Abstract: Antidepressants increase neurogenesis in adult dentate gyrus (DG), which is considered to be involved in the therapeutic action of antidepressants. However, the mechanism underlying it remains unclear. Using the culture system of adult rat DG-derived neural precursor cells (ADP), we have already reported that antidepressants have no direct effects on ADP. Therefore, antidepressants may increase neurogenesis in adult DG with unknown indirect mechanisms. We have also reported that amitriptyline (AMI), a tricyclic antidepressant, increases the secretion of GDNF, BDNF, FGF2 and VEGF from primary cultured astrocytes (PCA). All of GDNF, BDNF, FGF2 and VEGF increase in vivo neurogenesis in DG of adult rodents. These suggest that AMI-induced factors in astrocytes may increase neural precursor cells in adult DG. To examine this hypothesis, we examined the effects of GDNF, BDNF, FGF2, VEGF and conditioned medium (CM) from PCA treated with AMI on ADP proliferation.
ADP were prepared from adult DG of adult rat and cultured with Neurobasal-based medium. PCA were prepared from hippocampus of postnatal rat and cultured with DMEM-based medium. CM was prepared by culturing PCA with Neurobasal-based medium. When the effects of CM on ADP proliferation, CM and Neurobasal-based medium were equally mixed. The effects of CM, factors and drugs on ADP proliferation were examined with BrdU immunostaining.
AMI had no direct effect on ADP proliferation, but AMI-treated CM increased ADP proliferation in response to concentrations of AMI treated in PCA. Thus, AMI may indirectly increase ADP proliferation through inducing BDNF, GDNF, FGF2 and/or VEGF from PCA. Next, the expression of the receptors of BDNF, GDNF, FGF2 and VEGF in ADP were examined with RT-PCR. The receptors of GDNF, BDNF and FGF2, but not VEGF, were expressed in ADP. Following it, the direct effects of BDNF, GDNF and FGF2 on ADP proliferation were examined. Only FGF2 significantly increased ADP proliferation. To confirm that AMI-induced FGF2 surely mediates the increasing effects of AMI-treated CM on ADP proliferation, the effects of SU5402, a specific inhibitor of FGF receptors and anti-FGF2 antibody on ADP proliferation were examined. Both SU5402 and anti-FGF2 antibody significantly canceled the increasing effects of AMI-treated CM on ADP proliferation.
Our present study has shown that AMI indirectly increases ADP proliferation via inducing FGF2 secretion from PCA. FGF2 in brain is mainly derived from astrocytes. Astrocyte is a key component of the neurogenic niches in adult DG. Therefore, antidepressants may increase in vivo neurogenesis in adult DG through inducing FGF2 secretion from astrocytes.

Disclosures: S. Boku: None. **K. Hisaoka-Nakashima:** None. **S. Nakagawa:** None. **A. Kato:** None. **N. Kajitani:** None. **T. Inoue:** None. **I. Kusumi:** None. **M. Takebayashi:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.04/EE18

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: GABAb receptor in the ventral pallidum and its implication in depressive-like behaviors

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Abstract: Major depressive disorder is an idiopathic syndrome characterized by depressed mood, helplessness, anhedonia, disphoria, altered sleep behavior and appetite, loss of concentration and sometimes is associated with suicide. It has been reported that GABA release in the ventral pallidum (VP) is increased during depressive-like behaviors and the GABAA receptor seems to mediate a tonic instead of a phasic inhibition. However, the function of the GABAB receptor in the VP is not clear. We studied the effect of a specific GABAB agonist baclofen (25, 50 and 100 ng/side) and the antagonist saclofen (100 ng/side), bilaterally microinjected in the VP during the forced swim test (FST) in rats. Intra-VP microinjections of baclofen before FST significantly reduced swimming behaviors (172.1 ± 65.5; n=10, F(4;450), p=0,040) while saclofen did not produce any behavioral change (249.9 ± 161.0, n=10, F(0;001); p=0,970) compared to the control group saline solution (251.1 ± 98.6, n=10) during the day-2 of the FST. On the contrary, saclofen seems to enhance baclofen effect in reducing swimming times (115.83±16.6, n=6, F (16; 238); p=0,001) when both drugs were combined. No significant changes in the swimming behaviors were found between any group (experimental and control)during the day-1 on the FST. Open field test demonstrated that neither baclofen nor saclofen altered locomotor activity so the changes registered during FST could be attributed to modulation of the depression-like behavior. In a separate experiment we measured mRNA expression in the VP of both GABAB receptor subunits (GABAB1 and GABAB2) using semi quantitative RT-PCR comparing rats that showed depressive-like behaviors versus a control group. None changes were found for neither of the subunits GABAB1 and GABAB2. These results suggest that GABAB receptor on the VP might be related to depression-like behaviors and might be acting in a phasic inhibition without any changes in mRNA expression of both of its subunits. Baclofen emulates the increase GABAergic tone reported on the VP during FST and it decreases significantly swimming times as important symptom of behavioral despair.

Disclosures: R.E. Contreras: None. **M. Skirzewski:** None. **L. Betancourt:** None. **L. Hernández:** None. **P. Rada:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.05/FF1

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Title: Induction of CaMKIIβ by HDAC inhibitors might be involved in structural plasticity and behavioral responses to chronic stress

Authors: *T. HOBARA[1], S. UCHIDA[1], H. YAMAGATA[1], F. HIGUCHI[1], N. HIGUCHI[1], T. SHIBATA[1], K. OTSUKI[1], Y. WATANABE[2];
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Abstract: Recent reports have suggested that epigenetic gene regulations are closely associated with the development of stress vulnerability, and also contribute to behavioral responses to chronic stress and antidepressants. There is evidence suggesting that histone deacetylase (HDAC) inhibitors have antidepressant-like effects in rodents. However, molecular mechanisms of antidepressant actions induced by HDAC inhibitors remain unclear. The purpose of this study is to clarify molecular mechanisms of antidepressant actions by HDAC inhibitors in vivo and in vitro. First, we confirmed the antidepressant effect of HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) using model mice of depression. We found that subchronic treatment with SAHA reversed the increased depression-related behaviors in mice subjected to chronic stress. In addition, subchronic treatment with SAHA enhanced the expression of calcium/calmodulin-dependent protein kinase (CaMK) IIβ mRNA in the hippocampus of stressed mice. However, subchronic treatment with imipramine, a tricyclic antidepressant , did not affect the expression of CaMKIIβ as well as depression-related behaviors in stressed mice. These data suggest that SAHA has rapid antidepressant actions, and that the induction of CaMKIIβ by SAHA may contribute to the antidepressant actions. We also found that subchronic treatment with SAHA enhanced adult neurogenesis in the dentate gyrus (DG) of the hippocampus. Our results showed that doublecortin positive immature neurons displayed increased dendritic arborization after subchronic treatment with SAHA. In addition, mice with

partially impaired neurogenesis by methylazoxymethanol acetate (MAM) demonstrated depression-related behaviors. Notably, SAHA recovered the increased depression-related behaviors induced by MAM via stimulating maturation of immature neurons. On the other hand, full ablation of neurogenesis by Cytosine β-D-arabinofuranoside hydrochloride (AraC) demonstrated sustained depression-related behaviors in spite of co-treatment with SAHA. Furthermore, cell culture experiments showed the induction of CaMKIIβ mRNA and the increased number of differentiated cells by HDAC inhibitors. Importantly, CaMKIIβ knockdown inhibited the induction of cell differentiation by SAHA. Thus our data suggest that the induction of CaMKIIβ by HDAC inhibitors might be involved in structural plasticity and subsequent behavioral responses to chronic stress.

Disclosures: **T. Hobara:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.. **S. Uchida:** None. **H. Yamagata:** None. **F. Higuchi:** None. **N. Higuchi:** None. **T. Shibata:** None. **K. Otsuki:** None. **Y. Watanabe:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.06/FF2

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: KAKENHI 20452097

KAKENHI 24791208

KAKENHI 25860994

Title: Individual differences in pre-stress state predict distinct responses to chronic unpredictable stress in rats

Authors: *Y. IGUCHI, S. KOSUGI, Y. MINABE, S. TODA;
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Abstract: The individual differences among the patients with major depressive disorder (MDD) are frequently observed in symptoms and responses to antidepressants, suggesting the heterogeneity of this disease.However, the molecular mechanisms and/or genetic backgrounds

underlying them remain elusive. Preclinical models of MDD also have been facing the similar problems. We hypothesized that the pre-existing individual differences in various indexes such as motivation, flexible decision-making or endogenous stress-coping system in naive rats would work as predictive endophenotypes leading to distinct pathophysiological outcomes after the exposure to chronic unpredictable stress (CUS) that is widely used to induce MDD-like phenotypes in animals. To verify this hypothesis, we first tried to classify some distinctive subgroups from naive Sprague-Dawley rats (n = 84) by monitoring the differences in performance of motivation-dependent, goal-directed behaviors in a saccharin-rewarded progressive ratio instrumental training. As a result, the original population was divided into three subgroups as follows; Low Motivation (LM: characterized by consistent low completed ratio, 17% of total animals), Flexible (FL: high completed ratio in the first session followed by a drastic reduction, 57%), and Hyper Motivation (HM: consistent high completed ratio, 26%). The basal level of serum corticosterone was markedly higher in HM than in LM, and the augmentation of serum corticosterone in response to footstock was significantly higher in LM than in other subgroups. Next, we exposed all animals to CUS (randomized one of three stressors once a day; restraint, forced swimming, and social defeat) for 4 weeks, and then examined if these subgroups would differentially perform instrumental behavior after CUS. We found that, on the next day of the last session of CUS, the response rate on a continuous reinforcement schedule was accelerated by CUS in all subgroups. In the progressive ratio performance that was monitored every week, HM showed a significant reduction in the completed ratio immediately after CUS whereas LM showed a significant increase in the ratio 4 weeks after CUS. Meanwhile, the completed ratio was hardly affected in FL during the entire post-CUS period, implying relatively higher resilience to CUS in FL. These results suggested a predictability of distinct MDD-like phenotypes for each individual after CUS based on the individual differences in pre-stress state.

Disclosures: Y. Iguchi: None. **S. Kosugi:** None. **Y. Minabe:** None. **S. Toda:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.07/FF3

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: ANR-08-MNP-018 MCHPRIMAPARK

Biothèque Primate - Centre National de la Recherche Scientifique Life Sciences Department

Motac Neuroscience Ltd

Title: Toward a spontaneous model of depressive symptoms among cynomolgus and rhesus monkeys in farming conditions

Authors: *S. CAMUS[1], C. ROCHAIS[2], C. BLOIS-HEULIN[2], Q. LI[4], M. HAUSBERGER[3], E. BEZARD[1];
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Abstract: Although the pathophysiology of depressive disorders is extensively investigated, their underlying mechanisms remain poorly understood. Such studies have almost exclusively been performed in rodents submitted to environmental, pharmacological, surgical or genetic manipulations, i.e. induced model as opposed to spontaneously occurring disorders, therefore carrying poor construct validity. Adverse early-life experience might lead to the spontaneous expression of abnormal behaviors in animals and the predisposition to psychiatric disorder in Humans. Common breeding processes employ weaning and housing conditions different from what occurs in the wild. Thus, we investigated the existence of spontaneous atypical behaviors displayed by non-human primates (NHP), phylogenetically and behaviorally closer to Humans than rodents, in breeding farms and their possible similarities with human depressive symptoms. Following the identification of atypical behavioral- and their associated physiological-profiles among single-housed monkeys (n=80), we detected such profiles in more naturalistic settings (i.e. social housing) among 115 monkeys. We broached the potential impact of species and early-life experience on the prevalence of depressive-like profiles by analysing data from captive- or wild-born rhesus and cynomolgus macaques. Behaviors, body postures, body orientations, gaze directions, distances between individuals and locations in the cage were collected using an unbiased ethological scan-sampling method followed by multifactorial correspondence and hierarchical clustering analyses. In each species, housing, or origin conditions, we identified distinct profiles, one of them mimicking several depressive symptoms with high level of inactivity, facing the wall, low levels of locomotion, maintenance and investigation behaviors. A greater amount of depressive-like subjects were found in the captive-born and in the rhesus populations, suggesting that unnatural early life events increase the risk of developing pathological symptoms and that rhesus might be more vulnerable to express such symptoms compared to cynomolgus monkeys.
Both birth origin and species should be considered when setting up a preclinical research protocol. The use of unbiased behavioral observations might allow the identification of animal models of human mental/behavioral disorders and their most appropriate control groups.

Disclosures: S. Camus: None. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac Neuroscience Ltd. **C. Rochais:** None. **M. Hausberger:** None. **C. Blois-heulin:** None. **Q. Li:** F. Consulting Fees (e.g., advisory boards); Motac Neuroscience Ltd.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.08/FF4

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Characterization of the learned helplessness model and effects of P2X7 blockade

Authors: *N. C. WELTY, M. MORTON, J. SHELTON, B. SAVALL, M. LETAVIC, A. BHATTACHARYA, G. CHEN, J. R. SHOBLOCK;
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Abstract: The learned helplessness paradigm is a model of depression in which animals are exposed to inescapable shock (IES) and subsequently demonstrate a deficit in evading an escapable shock. Rats that were exposed to inescapable shocks (1.0mA, 15 sec, 120) showed an increase in plasma corticosterone that may have remained chronically elevated compared to animals that were naïve to shock. Altered glucocorticoid receptor expression in the prefrontal cortex and hypothalamus were also observed in animals subjected to shock, which may contribute to some of the behavioral deficits seen in this model. Interestingly, stress resilient animals appeared to have downregulated glucocorticoid receptor in hippocampus. Our preliminary data also suggest that two days after IES, iNOS, capsase-3, and Bax mRNA were all upregulated, suggesting excitotoxicity and apoptosis could be another driving factor of the observed deficits in this model. Since P2X7 is reported to be involved in stress adaptations, glutamate release, and apoptosis, we hypothesized that P2X7 antagonists would be effective in this model. We tested the effects of a P2X7 antagonist (Compound A, 10 mg/kg, s.c.) in the learned helplessness paradigm, dosed 1 h prior to the IES and 1 h prior to testing, for escape deficits and female urine sniffing deficits, a measure of anhedonic-like activity. The P2X7 antagonist had no effect on escape deficits, but appeared to at least partially attenuate the sniffing deficit caused by IES. P2X7 blockade may prevent the development of the anhedonic-like state following IES, perhaps by attenuating IES-induced excitotoxicity or HPA-axis dysregulation. Future experiments to confirm are pending.

Disclosures: N.C. Welty: None. **M. Morton:** None. **J. Shelton:** None. **B. Savall:** None. **M. Letavic:** None. **A. Bhattacharya:** None. **G. Chen:** None. **J.R. Shoblock:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.09/FF5

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Swedish Research Council

Karolinksa Institutet Funds

Lars Hiertas Minne Foundation

Title: Global, early, selective and progressive noradrenergic axonal degeneration in somatostatin 2 receptor but not in somatostain 1 receptor knockout mice

Authors: *C. ADORI[1], L. GLUECK[2], T. YOSHITAKE[3], J. KEHR[3], H. TOMAS[3];
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Abstract: The somatostatin peptide (SST) has been originally described as a growth hormone release-inhibiting factor but also considered as a neuromodulatory agent in the CNS. SST has an influence on, among others, pain mechanisms, REM sleep and brain ontogenesis. Changes in SST levels and in its receptor expression have been associated with dementia, epilepsy or major affective disorders. SST acts on six types of G-protein-coupled receptors in the brain. Among them, the inhibitory receptor SST2 is the most abundant. In SST2 knockout (KO) mice, increased anxiety-related behavior was described in a number of behavioral paradigms, while locomotor and exploratory activities were decreased in stress-inducing situations. However, no morphological study examined the brains of SST2 KO mice extensively. In our present work we looked for potential alterations in the brain chemical neuroanatomy of SST2 and SST1 KO animals.
E18 - 8 month old SST2 ko /LacZ knockin and SST1 ko /LacZ knockin mice were perfused for fluorescence immunohistochemistry and double-labelings (SST2a receptor plus monoaminergic or cholinergic markers, β-galactosidase, CCK, cortical interneuron- and pyramidal cell markers or glial markers) or were decapitated, and the brains were processed for in situ hybridization (tyrosine hydroxylase, galanin, CCK), HPLC measurements (monoamines and their metabolites) and radioimmunoassay (CCK, somatostatin). The spatial and temporal patterns of noradrenergic alterations, the number of locus coeruleus (LC) cell bodies and their soma area were determined by morphometrical techniques.
We found a widespread and progressive noradrenergic degeneration in SST2 KO, but not in

SST1 KO mice. We showed that this degeneration was selective for the noradrenergic system and apparently did not affect dopaminergic, serotonergic or cholinergic systems. While the LC cell bodies were seemingly intact even in the 8 month old animals, an abnormal noradrenergic fiber pattern could be detected already in the late prenatal period (E18). In addition, we also observed a considerable decrease of CCK immunostainig, decreased CCK mRNA expression and some signs of mild degeneration of CCK immunoreactive fibers in the neocortex, hippocampus and striatum of SST2 but, again, not in SST1 KO animals.
Our results indicate a fundamental role of the SST2 receptor in the global maintenance and integrity of the CNS noradrenergic system. To what extent similar degenerative/expression changes may occur in any neuropsychiatric/neurodegenerative disorder is an interesting question. If so, our studies may provide critical data for therapeutic intervention involving, presumably, SST2 agonists.

Disclosures: C. Adori: None. **L. Glueck:** None. **T. Yoshitake:** None. **J. Kehr:** None. **H. Tomas:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.10/FF6

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH 1R01MH090264

J&J/IMHRO Rising Star Translational Research Award

NARSAD Young Investigator Award

Title: Individual differences in peripheral inflammatory signaling controls susceptibility to social defeat stress

Authors: *G. E. HODES[1], M. PFAU[1], S. A. GOLDEN[1], D. J. CHRISTOFFEL[1], M. HESHMATI[1], H. ALEYASIN[1], M. LEBOEUF[2], M. MERAD[2], S. J. RUSSO[1];
[1]Neurosci., [2]Gene and Cell Med. and Oncological Sci., Icahn Sch. of Med. at Mt. Sinai, New York, NY

Abstract: Interleukin-6 (IL-6) is increased in the blood of subjects with depression and may reflect hyperactivity of the peripheral immune system. We utilized repeated social defeat stress (RSDS), an animal model of depression, to examine individual differences in the peripheral

immune response to stress. After exposure to RSDS some animals termed susceptible show a spectrum of depression-like behavior, whereas resilient animals behave more akin to controls. Susceptible mice exhibit heightened IL-6 levels following their first defeat, which remains elevated 48 hours after the last defeat. To examine if IL-6 levels contribute to individual differences in stress sensitivity, peripheral mononuclear cells (PBMCs) were isolated before mice were exposed to RSDS. Cells were stimulated with lipopolysaccharide (LPS) in vitro to examine IL-6 release. PBMCs from animals who later developed a susceptible phenotype had an exaggerated release of IL-6 in response to LPS stimulation. IL-6 release negatively correlated with social interaction ratio scores. Furthermore, mice that became susceptible to RSDS had more circulating PBMCs than mice that displayed a resilient phenotype. The number of isolated PBMCs also negatively correlated with the individual animals social interaction ratio score. To test the functional relevance of what appears to be a heightened peripheral IL-6 response to stress, we blocked susceptibility to RSDS by systemically injecting an antibody that neutralized IL-6 in the periphery. Additionally, IL-6 knockout mice also displayed resilient behavior. We then ablated the peripheral immune system of C57BL/6J mice and replaced it with bone marrow from IL-6 knockout mice. Bone marrow transplantation from an IL-6 knockout mouse promoted resiliency to RSDS. Furthermore, bone marrow transplants from susceptible mice induced social avoidance following a sub-threshold microdefeat. Together these studies indicate that individual differences in the inflammatory response to stress underlie the development of depression-like behavior in the social defeat model.

Disclosures: G.E. Hodes: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Johnson & Johnson. **M. Pfau:** None. **S.A. Golden:** None. **D.J. Christoffel:** None. **M. Heshmati:** None. **H. Aleyasin:** None. **M. Leboeuf:** None. **M. Merad:** None. **S.J. Russo:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Johnson & Johnson.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.11/FF7

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: JSPS KAKENHI Grant 25460096

Title: The Role of 5-HT4 receptor in hippocampal neurogenesis increased by chronic SSRI treatment

Authors: *E. SEGI-NISHIDA[1], Y. IMOTO[1], T. KIRA[1], K. KOBAYASHI[2];
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Abstract: Hippocampal neurogenesis is increased by chronic treatment of selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs). SSRIs increase proliferation and differentiation of neural progenitors in the dentate gyrus (DG). Among 14 subtypes of 5-HT receptors, Gs-coupled receptors, such as 5-HT type 4, 5, 6, 7 receptors, in the postsynaptic cells have been postulated to be involved in the promotion of neurogenesis. Since 5-HT type 4 (5-HT4) receptor is expressed at a high level in the DG and the treatment of 5-HT4 receptor agonist increases cell proliferation in the DG, we investigated whether 5-HT4 receptor is involved in the promotion of hippocampal neurogenesis by SSRI treatment by using 5-HT4 receptor deficient (5-HT4R KO) mice. 5-HT4R KO mice and wild-type (WT) mice were treated with fluoxetine as a SSRI at a dose of 22 mg/kg/day for 21 days. While the fluoxetine treatment significantly increased cell proliferation in the DG of WT mice, no significant change was observed between saline and the fluoxetine treatment in 5-HT4R KO mice. Doublecortin-immunoreactivities (DCX-IRs), as one of the markers for neurogenesis, in the DG were also increased in fluoxetine-treated WT mice compared with saline-treated WT mice. On the other hand, no changes were observed in DCX-IRs between saline and the fluoxetine treatment in 5-HT4R KO mice. We then examined whether 5-HT4 receptor regulates expression change of genes by the fluoxetine treatment in the DG. The expression of several genes, such as NPY, in the DG of WT mice was significantly increased by the fluoxetine treatment, but not in that of 5-HT4R KO mice. These results suggest the involvement of 5-HT4 receptors in the increase of the hippocampal neurogenesis by the chronic SSRI treatment.

Disclosures: E. Segi-Nishida: None. **Y. Imoto:** None. **T. Kira:** None. **K. Kobayashi:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.12/FF8

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Epigenetic modulation of hippocampal mGluR5 regulates coping strategies to repetitive stress

Authors: *Y. YEONG SHIN, G.-T. KIM, C. KIM, D. KIM;
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Abstract: After repetitive exposure to the same stressor, varied behavioral responses can occur and are often maintained long time. We investigated the role of mGluR5 in individual differences in coping strategies established in response to stressful stimuli in rats and discovered that, repeated exposure to restraint stress caused variably altered mGluR5 protein expression levels in the hippocampus. SD rats were subjected to the restraint stress for 1 hour/day for 6 consecutive days, and the effect of restraint stress on mGluR5 expression was examined. During each stress episode, electroencephalogram (EEG) was measured in order to observe the real-time brain activity. The low mGluR5 protein expression group showed increased methylation sites on the CpG island of the mGluR5 gene, decreased mGluR5 mRNA expression, and unaltered basal theta electroencephalogram power and corticosterone blood concentrations, suggesting positive behavioral adaptation. In contrast, the high mGluR5 protein expression group showed the opposite results, suggesting negative adaptation. These individual differences were abolished by injection with the mGluR5 antagonist MPEP. Thus, this study suggests that memories on previous experiences of stress determines levels of individual's stress controllability, which then constructs certain skills for behavioral adaptation needed to cope with stress when given repetitively. Moreover, this study also found that mGluR5, one of the candidate molecules that may have potential connections to behavior adaptation patterns, played a critical role in the development of these stress coping strategies.

Disclosures: Y. Yeong shin: None. **G. kim:** None. **C. kim:** None. **D. kim:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.13/FF9

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH090574

Title: Next generation sequence analysis of mRNA expression in the prefrontal cortex of mice subjected to chronic unpredictable stress

Authors: *J. A. AZEVEDO[1], C. L. COOKE[2], J. M. MCKLVEEN[4], J. P. HERMAN[4], R. C. THOMPSON[3];
[1]PiBS Neurosci., [3]Psychiatry, [2]Univ. of Michigan, Ann Arbor, MI; [4]Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Chronic stress is recognized as a major precipitating event for a host of psychiatric illnesses including affective and mood disorders. Here we report the use of unbiased next-

generation sequencing methods to examine mRNA expression profiles in the infralimbic/prelimbic cortex (IL/PL) of mice subjected to chronic variable stress (CVS), an animal model of depression, as well as control mice.
For mRNA expression profiling, RNA libraries were constructed using Illumina adapters and methods. Sequences were generated by the University of Michigan DNA sequencing core facility with an average of 57.7 million reads per sample. After sequences were confirmed to contain inserts, adapter sequences were trimmed and the resulting 50 base pair reads mapped to UCSC genome annotations. Final mRNA counts were derived through an Oracle-based pipeline and subjected to RPKM normalization to derive the final results.
Analysis revealed roughly 5,000 differentially expressed transcripts ($p<0.05$). We selected 64 mRNAs for qPCR validation (60 transcripts identified by RNA-seq as differentially expressed, 2 endogenous controls and 2 “housekeeping” genes) based primarily on prior association with psychiatric illness. The expression of these transcripts were then measured using Taqman Low-Density Array cards (ABI); from these, 31 transcripts achieved significant differential expression compared to controls ($p<0.05$).
Comparative in situhybridization studies currently underway focus on a subset of these mRNA transcripts to identify the neuroanatomical component of stress-induced mRNA alterations. In addition, as prior studies have linked microRNA (miRNA) disruption to both anxiety and affective disorders (Perkins et al, 2007; Kim et al, 2011) we are currently performing additional miRNA profiling studies to identify stress-induced alterations in the miRNA regulatory network. By examining widespread genomic disruptions, as well as their neuroanatomy and epigenomic regulatory effects, we will be in a position to better understand the contribution of dysregulated gene expression in the pathophysiology of psychiatric illness.

Disclosures: J.A. Azevedo: None. **C.L. Cooke:** None. **J.M. McKlveen:** None. **J.P. Herman:** None. **R.C. Thompson:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.14/FF10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Institute for the Study of Affective Neuroscience (ISAN) Grant

Title: Comparing chronic exposure to light and agomelatine in the reversal of depressive-like behaviour in a rat model of depression

Authors: *J. J. DIMATELIS[1,2], D. J. STEIN[3], V. A. RUSSELL[2];
[2]Human Biol., [3]Psychiatry and Mental Hlth., [1]Univ. of Cape Town, Cape Town, South Africa

Abstract: Exposure to stressors during the early stages of development has been shown to have a negative impact on psychopathology in later life. Rat pups subjected to repeated separation from the dam (for 3h per day during the first 2 weeks of life) display behavioral, endocrine and molecular changes that reflect changes found in patients with depression and hence provides a useful model to study the molecular basis of behavioral alterations induced by early adversity. Light treatment has previously been shown to have antidepressant effects in a rodent model of maternal separation (MS) and hence this study aimed to compare the effects of chronic constant light exposure (3 weeks, from postnatal day (P) 42 - 63 during adolescence with an anti-depressant (agomelatine, treated from P42 - 99) in MS rats. As agomelatine acts on similar molecular targets as light exposure, the comparison between treatments would provide valuable information with regard to their mechanism of antidepressant action. Behavioral assessments included ultrasonic vocalizations (22 kHz) and forced swim test (FST) which were conducted at two time points (P65-67 and P97-99) to determine short and long term effects of treatment. At P98, MS resulted in an overall increase in the duration of 22 kHz vocalization, which is in line with increased anxiety-like behavior in depressed animals. At P65 and P98, treatment with agomelatine resulted in increased duration of 22 kHz calls in MS animals compared to vehicle treated rats. Similarly, agomelatine increased the number of 22 kHz calls at P65 of MS rats. Light treatment did not have an effect on 22 kHz vocalizations at any time point. No differences were found in locomotor activity in the open field. In the FST, MS resulted in decreased swimming and hence were less mobile, while agomelatine increased swimming in MS rats, which is in line with the molecular actions of the antidepressant acting to increase serotonergic neurotransmission and that increased swimming in the FST is driven by a greater serotonergic drive. Conversely, MS increased climbing in the FST, while agomelatine decreased climbing behavior in MS rats. The results suggest that agomelatine has an anxiolytic/antidepressant effect on MS rats. Agomelatine also appeared to increase social anxiety and exploratory activity in the MS rats by increasing 22 kHz calls when removed from the home cage and increasing time spent in the center zone of the open field. Constant light exposure during adolescence did not have any effect in this study.

Disclosures: J.J. Dimatelis: None. **D.J. Stein:** None. **V.A. Russell:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.15/GG1

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Involvement of homer1a in resilience to chronic mild stress

Authors: *Y. SHUI[1,2], R. YAMAMOTO[2], N. KATO[2];
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Abstract: Chronic stress is a risk factor for psychiatric diseases. Homer1a is an activity-dependently induced member of the scaffold protein family Homer1, which is implicated in stress-induced emotional changes. To clarify the role of Homer1a protein during chronic mild stress, we investigated the effects of chronic restraint stress (CRS) on wild-type and Homer1a knockout (Homer1a(-/-)) mice. Both types of mice were under restraint for 2 hrs daily for 7 consecutive days, and compared with respective no-restraint controls in behavioral tests. In either group of mice, the restraint induced no change in anxiety-like behavior or pain responses. However, the open field test revealed hypoactive locomotion in the Homer1a (-/-) group without restraint, whereas those with restraint showed a significantly elevated locomotive activity. In brain slices prepared from the mice used for behavior, we examined neuronal excitability in cingulate cortex pyramidal neurons and synaptic efficacy in the hippocampal CA1. In wild-type mice after restraint, the excitability or synaptic efficacywas not significantly different than in non-restraint controls. By contrast, in slices from Homer1a (-/-) mice after restraint, the excitability was elevated as assessed with the frequency of spikes evoked by current injection. Also, the restraint broadened the spike width, which was caused byBK channel blockade. Furthermore, the synaptic efficiency was significantly increased, as revealed by calculating the fEPSP slope vs. the size of fiber volley. These results suggest that the locomotive activity increased by chronic mild stress in Homer1a (-/-) mice may be attributed to cortical hyper-excitability and synaptic up-regulation, and that Homer1a may be required for resilience to chronic mild stress.

Disclosures: Y. Shui: None. **R. Yamamoto:** None. **N. Kato:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.16/GG2

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: N.I.H.

Falk Medical Research Trust

Title: Nogo-Receptor-1 limits behavioral and anatomical plasticity associated with fear learning and extinction

Authors: *S. M. BHAGAT[1], S. M. STRITTMATTER[2];
[1]Cell. Neuroscience, Neurodegeneration and Repair, [2]Neurol., Yale Univ., New Haven, CT

Abstract: During early development behavioral and cortical plasticity are more labile compared to adulthood. Previous studies have shown that extinction training can erase fear memories in rodent pups (prior to P16). However, in adulthood, after the formation of myelin and perineuronal nets, fear memories become resistant to erasure. Our lab has recently shown that Nogo Receptor 1 (NgR1), a neuronal receptor for myelin associated inhibitors, limits cortical plasticity, decreasing turnover of dendritic spines and axonal varicosities in the adult CNS. We found that NgR1 knockout (KO) mice maintain juvenile levels of cortical plasticity throughout adulthood. Therefore, we are currently investigating whether adult NgR1 KO mice show similar behavioral plasticity in fear conditioning and extinction similar to that seen in juvenile mice. We found that adult male NgR1 KO mice show enhanced fear learning in a weak fear-conditioning paradigm compared to WT mice. In this paradigm mice are presented with three pairs of a tone followed by a low amplitude foot shock. However, with a stronger conditioning paradigm, including a higher amplitude foot shock, the NgR1 KO and WT mice showed equal freezing rates. On the second day of extinction, 48 hours after acquisition using the stronger fear conditioning paradigm, NgR1 KO showed enhanced extinction compared to controls. A week later we found that, unlike WT mice, NgR1 KO mice exhibit no spontaneous fear recovery during recall. This suggests that NgR1 KO adult mice undergo fear erasure following extinction. In addition, tamoxifen-inducible conditional NgR1 KO mice show similar rates of extinction and extinction recall as do constitutive NgR1 KO mice, indicating that these effects do not require NgR1 deletion during development. We are using two-photon in vivo imaging to explore the correlation of spine turnover in the frontal association cortex with the behavioral findings. These studies elucidate NgR1 as a potential novel target for treating Post Traumatic Stress Disorder.

Disclosures: S.M. Bhagat: None. **S.M. Strittmatter:** Other; Cofounder of Axerion Therapeutics, seeking to develop PrP- and NgR- based therapeutics.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.17/GG3

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Coast Capital Savings Depression Research Fund Young Investigator Grant

Title: Fluoxetine reverses disrupted maternal care but not depressive-like behavior after chronic corticosterone exposure

Authors: *J. L. WORKMAN[1], A. R. GOBINATH[1], N. F. KITAY[1], C. CHOW[1], S. BRUMMELTE[2], L. A. M. GALEA[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Wayne State Univ., Detroit, MI

Abstract: Women are twice as likely as men to suffer from depression and the postpartum period confers considerable risk for developing depression. Stress and glucocorticoids are consistently associated with depressive disorders and individuals with depression generally have higher cortisol concentrations and impaired HPA axis negative feedback. In many rodent studies, chronic variable stressors or exogenous corticosterone (CORT; the primary glucocorticoid in most rodents) induce a depressive-like phenotype. However, most models of depression have primarily used males. Our prior research indicates that high CORT given to postpartum females reduces maternal care, increases depression-like behavior, and alters hippocampal plasticity, consistent with a depressive-like phenotype. The goals of the present study were to investigate how chronic CORT alters behavior and the brain in females at different reproductive stages and to determine whether a CORT-induced behavioral phenotype can be reversed using a common antidepressant, fluoxetine (FLX). Female Sprague Dawley rats were either mated or remained reproductively inexperienced (nulliparous) and then received either CORT or oil and FLX or saline (yielding 8 groups) every day for 22 days (for postpartum rats, this coincided with postpartum days 2-24). Maternal care was observed postpartum days 2-8 and depressive-like behaviors were assessed in the forced swim test (FST) on days 23-24. As previously established, postpartum CORT disrupted maternal care and increased immobility in the FST. Notably, FLX reversed CORT-induced changes in maternal care. However, FLX did not significantly decrease immobility in the FST in either nulliparous or postpartum females. Furthermore, contrary to our expectations, nulliparous females spent more time immobile compared with postpartum females in response to CORT. Nulliparous females also had a shorter latency to first immobility. We are currently investigating how CORT and FLX regulate hippocampal plasticity in this study. These data contribute to our understanding of how reproductive states may alter the susceptibility to depression and antidepressant efficacy in CORT-induced depression-like behavior in females. Supported by Coast Capital Depression Fund.

Disclosures: J.L. Workman: None. **A.R. Gobinath:** None. **N.F. Kitay:** None. **C. Chow:** None. **S. Brummelte:** None. **L.A.M. Galea:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.18/GG4

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant 5T32NS069562-03

NIH Grant R01 MH070727

Title: The role of spontaneous neurotransmission in fast-acting antidepressant response

Authors: *E. S. GIDEONS, E. T. KAVALALI, L. M. MONTEGGIA;
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Abstract: Recent work has shown that spontaneous release of glutamate activates a subset of *N*-methyl-*D*-aspartate receptors (NMDARs), which are distinct from NMDARs that are activated by action-potential mediated release of glutamate. Spontaneous release of glutamate and activation of NMDARs generates relatively small influxes of calcium (Ca^{2+}) that has been shown to mediate tonic levels of eukaryotic elongation factor 2 (eEF2)-kinase activity in phosphorylating eEF2, thereby suppressing protein translation in the dendrite. In contrast, acute blockade of NMDARs at rest inhibits eEF2-kinase phosphorylation of eEF2; leading to desupression of local translation in the dendrite of many proteins. Ketamine, a non-competitive NMDAR antagonist, causes a fast-acting anti-depressant effect in depressed patients and preclinical models. Additionally, it causes decreased phosphorylation of eEF2 and increased BDNF translation in the hippocampus within 30 min following administration. While the clinical data of ketamine's fast-acting antidepressant effect is exciting, it is known that ketamine has a high abuse potential so memantine, a NMDAR open channel blocker that is FDA approved for other indications, is under investigation for fast-acting antidepressant efficacy. A single dose of memantine has not been shown to cause a fast-acting antidepressant effect in depressed patients leading to the natural question, why not? This study will address three main questions: 1) does a single dose of memantine cause a fast-acting antidepressant response in a preclinical mouse model? 2) does ketamine or memantine block the NMDAR at rest with physiological Mg^{2+} present? 3) what effects will memantine have on the phosphorylation of eEF2?

Disclosures: E.S. Gideons: None. **E.T. Kavalali:** None. **L.M. Monteggia:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.19/GG5

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: FAPESP Grant 11/51789-8

Title: Anxiety- and depressive-like behavior in an experimental model of perimenopause

Authors: *K. V. WEISSHEIMER[1], D. E. RIBEIRO[2], S. R. L. JOCA[3], J. A. ANSELMO-FRANCI[1];
[1]Dept. of Morphology, Physiol. and Basic Pathology, Univ. of Sao Paulo - Dent. Sch., Ribeirao Preto, Brazil; [2]Dept. of Pharmacol., Univ. of Sao Paulo - Sch. of Med., Ribeirao Preto, Brazil; [3]Dept. of Physics and Chem., Univ. of Sao Paulo - Sch. of Pharmaceut. Sci., Ribeirao Preto, Brazil

Abstract: Perimenopause is a stage of fluctuations in plasma levels of sex steroids, when the woman experiences regular and irregular cycles, or prolonged amenorrhea of less than 12 months. This transition period to menopause may cause an increase in vulnerability in some women to mood disturbances like depression and anxiety, resulting in a decreased quality of life. However there is some degree of controversy in the etiopathogeny of mood disorders in perimenopause. Hormonal, neurochemical and genetic factors have been suggested, but these explanations remain to be clarified. Ovariectomy has been the primary rodent model to examine effects of ovarian hormone loss on mood disorders. This model limits evaluations to abrupt and complete ovarian hormone loss, and it does not reproduce the transitional hormone fluctuation via ovarian follicular depletion that occurs in woman. An ovotoxic chemical, 4-Vinylcyclohexene-diepoxide (VCD), produces accelerated ovarian follicular depletion in rats, with reproductive hormone profiles similar to naturally menopausal transition in women. We have evidenced that VCD injected intraperitoneally (160 mg/kg/day) in rat for 15 days causes follicular depletion, decrease in progesterone levels and corporal temperature, 80 days after VCD treatment has begun, indicating a perimenopausal period. In this study, we investigated whether VCD administration would promote behavioral effects on mood of female rats and how they would respond to treatment with saline (S) or imipramine (Im), a tricyclic antidepressant. Rats (28 days) received vehicle (V) or VCD for 15 days. From the 45º day after this treatment, estrous cycles were verified during 2 weeks and animals were selected to the behavioral tests on diestrus II day within the regular cycle. Rats were submitted to open field and forced swimming test (FST) around the 80º day after the beginning VCD treatment. Open field was performed one day before FST. Animals received 3 injections of saline or imipramine (1 mg/kg) 24, 13 and 1 hour before FST. They were divided into V+S, VCD+S, V+Im and VCD+Im groups (n= 6-9/group). Open field and FST data were analyzed by t-test and two-way ANOVA respectively. Exploration of the periphery and rearing frequency of the open field were significantly reduced in VCD+S vs V+S. Immobility time in FST was significantly increased in VCD+S when compared to other

groups. Imipramine significantly decreased immobility time in FST in vehicle and VCD animals. Our results indicate that the VCD rat-model of perimenopause promoted mood disorders, similar to women during the menopause transition, and imipramine treatment prevented depressive behavior.

Disclosures: K.V. Weissheimer: None. **D.E. Ribeiro:** None. **S.R.L. Joca:** None. **J.A. Anselmo-Franci:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.20/GG6

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Effects of testosterone on depressive-like behavior and hippocampal gene expression in male rats

Authors: *S. K. SALAND[1,2], N. M. CARRIER[3], F. DUCLOT[1,2], M. KABBAJ[1,2];
[1]Dept. of Biomed. Sci. & Program in Neurosci., [2]Ctr. for Brain Repair, Florida State Univ., Tallahassee, FL; [3]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Gonadal hormones have been implicated in the pronounced sex differences in depression, where the prevalence is at least twice as high in women as it is in men. In particular, low levels of circulating testosterone in hypogonadal men are associated with an increased incidence of depression, which can be effectively reversed with testosterone replacement. Moreover, testosterone supplementation reverses and protects against depressive-like behaviors in gonadectomized (GNX) and aged male rats, respectively. Previous work from our lab demonstrated a role for the hippocampus, an area highly implicated in the etiology of depression, in mediating these effects in male rats. In this brain region, testosterone may exert its antidepressant effects through androgen receptor mediated mechanisms, or via local aromatization to estrogen. Therefore, we examined the roles of testosterone and estrogen on depressive-like behaviors and gene expression in the hippocampus of adult male rats. Supplementation with both testosterone and estradiol in GNX male rats attenuated depressive-like behavior in the forced swim test and sucrose preference test. Blocking the conversion of testosterone to estrogen in the hippocampus with the aromatase inhibitor fadrozole produced variable effects on behavior, suggesting a complex functional role of these hormones in the hippocampus on anxiety- and depressive-like behaviors in male rats. Genome-wide cDNA microarray analysis was used to examine potential mechanisms in the hippocampus by which

testosterone and estrogen influence depressive-like behavior, revealing substantial and overlapping transcriptional regulation of depression-relevant genes by both hormones in GNX male rats.

Disclosures: S.K. Saland: None. **N.M. Carrier:** None. **F. Duclot:** None. **M. Kabbaj:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.21/GG7

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Comparison of antidepressive effects following cortical versus auricular electroconvulsive stimulation in rats

Authors: W. THEILMANN[1,2], C. BRANDT[1,2], M. RHEIN[3], H. FRIELING[3,2], S. BLEICH[3,2], *W. LOSCHER[1,2];
[1]Dept. of Pharmacology, Toxicology, and Pharm., Univ. of Vet. Med. Hannover, Hannover, Germany; [2]Ctr. for Systems Neurosci., Hannover, Germany; [3]Dept. of Psychiatry, Social Psychiatry and Psychotherapy, Hannover Med. Sch., Hannover, Germany

Abstract: Major depressive disorders are one of the most common psychiatric illnesses in industrial nations. A very effective treatment of severe depression is the induction of controlled therapeutic seizures, which are known as electroconvulsive therapy (ECT). There are several experimental studies in which electroconvulsive shocks (ECS) are delivered to rodents via earclip (auricular) electrodes. This is not in line with the clinical procedure where seizures are induced via stimulation of cortical areas, using electrodes attached to the scalp. It is known from epilepsy research that the location as well as the type of stimulation has a crucial impact on the seizure event.
Aim: The aims of this study were to establish a model of ECS that is closer to clinical conditions and to compare the regulation of brain-derived neurotrophic factor (BDNF) after auricular and cortical stimulation.
Methods: Male Wistar (Janvier) rats were implanted with screw electrodes over the primary motor cortex. The animals received five ECS on consecutive days either via cortical or auricular stimulation. Stimulation strength was 7-10 mA for 1 sec with cortical and 70-85 mA for 0.5 sec with auricular stimulation, using a 100-Hz stimulus. A simultaneous EEG-recording allowed the evaluation of seizure severity and length according to the clinical procedure. A further group of animals was treated with citalopram (15 mg/kg) for 21 days. The forced swim test, sucrose

preference test, hyponeophagia test and the recording of ultrasonic vocalization were performed to reach conclusions about the antidepressant effect of ECS. Subsequently, changes in DNA methylation and protein expression of BDNF in the prefrontal cortex and hippocampus were analyzed.
Results: Both types of stimulation induced similar convulsive seizures of 18-21 sec duration as well as similar effects in the forced swim test. Furthermore, auricular stimulation was associated with adverse effects such as significant weight loss and increased occurrence of aversive 22 kHz calls, which was not observed with cortical ECS. Preliminary experiments on Bdnf DNA methylation in the prefrontal cortex BDNF expression did not indicate any differences between control and ECS-treated rats.
Outlook: This new established model for ECT is the basis for further studies in chronic models of depression in which it is planned to investigate mechanism of resistance to ECS.

Disclosures: W. Theilmann: None. **W. Loscher:** None. **C. Brandt:** None. **S. Bleich:** None. **H. Frieling:** None. **M. Rhein:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.22/GG8

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NWO-ALW grant #819.02.022

Title: Adaptive fitness; early life adversity improves adult stress coping in heterozygous serotonin transporter knockout rats

Authors: *R. VAN DER DOELEN[1], T. KOZICZ[2], J. HOMBERG[3];
[1]Cell. Animal Physiol., Radboud Univ. Nijmegen, Nijmegen, Netherlands; [2]Anat., [3]Cognitive Neurosci., Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands

Abstract: Early life adversity in the form of childhood abuse has been associated with increased risk for the development of psychiatric disorders like major depression. Recent reviews, however, have postulated the possibility that instead of a necessary pathological role of early life adversity rather the match or mismatch between the early and later (e.g. adolescent, adult) life environment could determine vulnerability to stress-related psychiatric disorders. Using the maternal separation paradigm as a model of early life stress, we tested serotonin transporter heterozygous and homozygous knockout and wild-type rats for their shock escape behavior, after previously being exposed to inescapable shock stress. The maternal separation group showed

decreased escape latencies, which was found to be most significant in heterozygous serotonin transporter knockout rats (doi: 10.1038/mp.2012.186). Our study therefore shows that a putative match between early life adversity and later life adversity leads to an adaptive stress escape response, particularly in subjects that are stress sensitive by genotype. Our results nuance the prevailing theory that 5-HTTLPR s-allele carriers have an increased risk to develop depression when exposed to ELS, and suggest that carrying the s-allele does not inevitably have negative consequences. Rather, the increased sensitivity of s-allele carriers to a respective match or mismatch between the early and adult life environment may govern their adaptive or maladaptive responses to stress.
Within our animal model we have found that early life stress and serotonin transporter gene variation interact to program basal activity of the adult hypothalamo-pituitary-adrenal axis. We furthermore found that this gene x environment programming is most profound at the level of the adrenal gland. At the meeting, we would like to propose a working hypothesis which is a synthesis of our behavioural and physiological findings.

Disclosures: R. Van Der Doelen: None. **T. Kozicz:** None. **J. Homberg:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.23/GG9

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: CONACYT CB-CB2011-167436Q

Title: Clinical doses of citalopram reduce immobility time in the forced swimming test in Wistar rats with low immobility, while reboxetine or amitriptyline modulates all female rats. Citalopram (10 mg/kg) lacks of effect in all female rats

Authors: *J. PINEDA, A.-G. FLORES-SERRANO, F. J. HEREDIA-LOPEZ, F. J. ALVAREZ-CERVERA, J. L. GONGORA-ALFARO;
Univ. Autonoma de Yucatán, Merida, Mexico

Abstract: The sensitivity of the immobility time (IT) in the forced swimming test to antidepressant-drugs such as Amitrityline differs in rats expressing motor activity with high or low level during the first five minutes at the onset of the conditioning session of the forced swimming test (FST). However, it is not known whether this heterogeneity is also expressed in female rats after the administration of the most selective reuptake inhibitors for 5-HT (SSRI) citalopram, or for noradrenaline (SNRI) reboxetine. Since it is known that the effective dose of

the SSRIs and SNRIs to modulate the behavior during the forced swim in rodents is about ten times larger than the dose used in clinical, in this work we compared the influence of clinical doses of either the SSRI citalopram or the SNRI reboxetine, with the action of the tricyclic antidepressant amitriptyline on two subgroups of female Wistar rats which expressed high IT (HI; at or above the mean) or low IT (LI; below the mean) during the initial 5 minutes of the first session of the FST. When vehicle was applied to any of HI or LI rats, the IT increased in the second session of the test ($p < 0.001$). This increment concurred with a climbing time (CT) decrement ($p < 0.001$) without any change in the swimming time (ST; $p > 0.05$). When amitriptyline (15 mg/kg) was tested, the CT increased for both HI and LI rats ($p < 0.0001$). This increment was accompanied by an IT decrement in HI rats ($p < 0.003$), while for LI rats there was no change in the ST ($p > 0.05$) or in the IT ($p > 0.05$). Reboxetine (0.16 or 1 mg/kg) precluded the IT and CT changes in both HI and LI rats ($p > 0.05$) and reduced the ST in LI rats ($p < 0.05$). Citalopram (0.4 to 3 mg/kg) differentially mimicked the influence of reboxetine on the IT and CT in LI rats ($p > 0.05$), and in HI rats only at 3 mg/kg ($p > 0.05$). The effect persisted when the IT and the CT of LI rats were evaluated with citalopram (3 mg/kg) during metaoestrus/diestrus, and during proestrus/estrus. Yet, at the dose of 10 mg/kg citalopram completely lacked of effect in HI or LI rats. None of the tested drugs increased motor activity in the open field test. These results show that clinical doses of citalopram selectively preclude to the behavioral changes only in female Wistar rats showing high level of motor activity at the onset of the first session (first five minutes) of the FST. Alternatively, the amitriptyline or reboxetine preclude the behavioral changes during the FST in all the female Wistar rats.

Disclosures: **J. Pineda:** A. Employment/Salary (full or part-time):; Universidad Autonoma de Yucatan. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CONACYT CB2011-167436Q. **A. Flores-Serrano:** None. **F.J. Heredia-Lopez:** None. **F.J. Alvarez-Cervera:** None. **J.L. Gongora-Alfaro:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.24/GG10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: The National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0006200)

Title: The change of astrocytic proteins in the depressive-like mouse brain

Authors: *H. KIM, H. G. S. S. HYUN JOON KIM;
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Abstract: Many evidences from postmortem and rodent studies indicate that astrocyte pathology and glutamate metabolism abnormalities are associacted with the aetiology of depression. Astrocyte is known to play an important role in the glutamate metabolism through forming tripartite synapse and glutamate/glutamine (Glu/Gln) cycle. The glutamine synthetase (GS), an enzyme that forms glutamine by an ATP-dependent amination of glutamate, is related to metabolism of neurotransmitter and the glial fibrillary acidic protein (GFAP) is relevant to structural change of astrocyte. Therefore, this study investigated the changes of GS and GFAP in the mouse brain after chronic immobilization stress (CIS), a rodent model of depression. Moreover, we measured Glu/Gln contents in the brain and blood using high-performance liquid chromatography. To measure the GS activity more directly, we established new GS activity assay method. Animals exposed to CIS exhibited a significant reduction in body weight and behavioral symptoms of depression in the sucrose preference test and forced swim test. The GS activity was increased in the prefrontal cortex (PFC) of CIS-induced depression animals, but not GS expression. Unlike the GS, GFAP was decreases in the PFC in stress group compared with the control. The Glu and Gln levels were decreased after CIS treatment in the PFC and blood. These results suggest the involvement of astrocytic glutamate regulation and structural alteration in the aetiology of depressive disorder.

Disclosures: H. Kim: None. **H.G.S.S. Hyun Joon Kim:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.25/GG11

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Female H/Rouen mice selectively bred for depressive-like behavior display enhanced vulnerability for cocaine reinforcing effects

Authors: *V. RAPPENEAU[1], A.-L. MOREL[1], P.-H. LUPPI[1], J.-M. VAUGEOIS[2], M. EL YACOUBI[1], A. BEROD[1];
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Abstract: Cocaine addiction is often associated with psychiatric disorders, in particular with depression. To shed light on the neurobiological mechanisms by which depression-like states may enhance addictive behaviors, we used Helpless mouse line (H/Rouen) that exhibit depression-like behavior in validated tests compared to Non Helpless mouse line (NH/Rouen) derived from the same colony.
To test the sensitivity to cocaine of these two lines, dose-response curves for cocaine-induced motor activity (0-30 mg/kg doses) were performed. Interestingly, a right shift of the dose-response curves and higher ED50 values were observed in both male and female H/Rouen mice, in comparison with NH/Rouen mice, indicating that H/Rouen mice were less sensitive than NH/Rouen mice to the acute psychomotor effects of cocaine. Interestingly, despite differences observed after acute injection, H/Rouen and NH/Rouen mice displayed similar sensitivity to chronic cocaine psychomotor stimulant effects assessed in the behavioral sensitization paradigm. Conditioned place preference (CPP) tests were then conducted to establish the relative potency of cocaine reinforcing effects in the different mouse lines. Preferences scores were evaluated 1 and 5 days after 4 cocaine pairings (10 mg/kg). In male mice, equivalent CPP responses were observed in the two lines. In contrast, female H/Rouen mice exhibited a stronger cocaine-induced CPP compared to NH/Rouen mice at 1 and 5 days after conditioning, indicating a higher sensitivity to cocaine reinforcing properties.
Our results show that psychostimulant properties of cocaine would not predict the strength of its reinforcing or addictive effects, suggesting that distinct neurobiological mechanisms underlie the motor and reinforcing effects of cocaine. Moreover, they offer the possibility to explore the neural substrates that mediate the increased sensitivity to cocaine reinforcing effects observed in female H/Rouen mice. Preliminary results, using Fos immunohistochemistry, highlight a lower activation of subregions of the prefrontal cortex in female H/Rouen mice compared to female NH/Rouen mice, suggesting a reduced executive control over motivation triggered by the cocaine-paired environment. Moreover, they underline a higher activation of the nucleus accumbens core and the amygdala in female H/Rouen mice compared to female NH/Rouen mice, that may reflect their higher propensity to conditioned drug-seeking. Further analyses are currently performed to fully characterize the neurobiological mechanisms underlying the enhanced vulnerability of H/Rouen females for rewarding effects of cocaine.

Disclosures: V. Rappeneau: None. **A. Morel:** None. **P. Luppi:** None. **J. Vaugeois:** None. **M. El yacoubi:** None. **A. Berod:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.26/GG12

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: PEst-C/SAU/UI3282/2011-COMPETE

Title: Prolonged depressive-like behavior in mice exposed to a bolus injection of methamphetamine

Authors: C. D. SILVA[1], I. PITA[1], A. F. NEVES[1], A. I. DIAS[1], H. J. FREITAS[1], S. M. MENDES[1], S. D. VIANA[1], P. A. DE OLIVEIRA[2], R. A. CUNHA[3], C. A. FONTES RIBEIRO[1], R. D. PREDIGER[2], *F. C. PEREIRA[1];
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Abstract: Mehamphetamine (METH) abuse leads to cognitive and mood abnormalities including depressive symptoms. A single METH-injection to rodents recapitulates the dopaminergic/serotonergic dysfunction seen in METH addicts. However, the behavioral correlate of these neurochemical deficits has been overlooked. Herein, we aimed to characterize the neurobehavioural changes at early and long-term stages following a single-high dose of METH. Adult C57BL/6 mice (3-4 month-old) were injected with 30 mg/kg METH,i.p and evaluated up to 49 days post-injection. Pole-test (PT), Morris water maze-(MWM, cued platform) and tail-suspension test (TST) were conducted to evaluate movement, procedural memory and depressive-like behaviors parameters. Frontal cortical and striatal changes in monoamine homeostasis as well as GFAP levels were also evaluated by HPLC-ED and western-blotting. All values were expressed as means ± SEM and statistical significance was evaluated by Student's two-tailed t test or by ANOVA followed by Newman-Keul´s (GraphPad Prism 5.00.288). The significance level was set at a p value less than 0.05. METH increased the immobility time at 3 and 49 days post-treatment in the TST ($p<0.05$). On the contrary, METH did not disrupt basal ganglia-related movement as seen in the PT at 3 days post-treatment. Additionally, METH did not impair procedural learning and memory over the four testing days in the cued version of the MWM (1-4 days post-treatment). Moreover, swimming velocity was not changed in METH treated mice. This further stresses that intoxicated mice had normal motor activity. As per neurochemistry parameters, DA and TH depletion (30 - 40%; $p<0.05$) that were observed in frontal cortex and striatum at 3 days remained at 49 days post-treatment. METH evoked a 5-HT depletion in frontal cortex at 3 days post-treatment (*circa* 25%; $p<0.05$) that was maintained for 7 weeks. A robust yet transient astrogliosis was observed in striatum at 3 days ($p<0.01$). This further confirms the neurotoxic profile of this single dose. Our results show for the first time that a bolus injection of methamphetamine imposes a prolonged depressive-like behavior underlined by a long-term serotoninergic/dopaminergic frontostriatal disruption. This experimental model of long-term depression-like behavior is of relevance to unravel the neurobiological substrate underlying depressive symptoms seen in METH addicts.
Supported by"Fundação para a Ciência e Tecnologia" (FCT) [PTDC/SAU-FCF/102958/2008]

under the frame of COMPTE and FEDER (FCOMP-01-0124-FEDER-011079) and by Strategy Project PEst-C/SAU/UI3282/2011-COMPETE

Disclosures: C.D. Silva: None. **R.A. Cunha:** None. **P.A. De Oliveira:** None. **R.D. Prediger:** None. **F.C. Pereira:** None. **I. Pita:** None. **A.F. Neves:** None. **A.I. Dias:** None. **S.D. Viana:** None. **C.A. Fontes Ribeiro:** None. **H.J. Freitas:** None. **S.M. Mendes:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.27/GG13

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: the National Natural Science Foundation of china 81171294

Title: Clomipramine relieves chronic social defeat induced depression-like symptoms in male tree shrews

Authors: *Y. X. YANG, SR, W. JING;
Kunming Inst. of Zoology, Yunnan, China

Abstract: In this paper, we use chronic social defeat model in male tree shrews (*Tupaia belangeri chinensis*) as a new depression animal model. Because activity-dependent persistent changes in synaptic strength can be mediated by stress processes in brain areas such as the hippocampus, we examined whether social defeat affected synaptic plasticity in the CA1 region of the hippocampus of stress-exposed tree shrews. We also tested the effects of a classical tricyclic antidepressant (TCA) clomipramine in this model to evaluate the face validity, the predictive validity, and the construct validity.
The modeling includes four phases: adapting phase (the 1^{st} week), stress phase (the $2^{nd}$to the 6^{th} week), drug administration with social defeat phase (the 3^{rd} to the 6^{th} week) and recovery phase (the 7^{th} week). Each two male tree shrews were housed in a pair-cage consisting of two independent cages separated by a wire mesh partition with a door connecting the two cages. After one week adaptation, the connecting door was opened and a brief fighting occurs between the two male tree shrews and this social conflict session consisted of 1 h direct conflict (fighting) and 23 h indirect influence (e.g. smell, visual cues) per day. The defeated tree shrew was considered the subordinate.After establishing the social defeat model successfully, we gave the drug intervention, and tested the symptoms correlated with the depressed patients (including anhedonia, urine cortisol level and negative memories) and the symptoms uncorrelated with the depressed patients (including body weight, jumping, self-grooming and avoid behavior) in each

weekend. Clomipramine was given 50 mg/kg intragastriclly (i.g.) every morning during the drug administration phase. It is important to note that the psychosocial stress situation was continued during the drug treatment. After 35 days chronic social defeat, depression-like symptoms as decrease of 5% sucrose preference, loss of body weight, reduction of locomotors and self-grooming, increase of avoidance behavior, disrupted urinary cortisol rhythm and strengthened of one-trial captive conditioning memory (that is negative memory) were all emerged on subordinate tree shrews (Sub + saline group) compared with naïve animals (Ctrl group).

Disclosures: Y.X. Yang: None. **W. Jing:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.28/GG14

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: The effect of maternal care on depressive-like behaviors in female offspring

Authors: *A. BORROW, M. STOLOW, N. M. CAMERON;
Psychology, Binghamton Univ., Binghamton, NY

Abstract: Natural variations in maternal care have demonstrated effects on progesterone levels in female rat offspring. Females exposed to low levels of maternal licking/grooming during the first week of life (Low LG) exhibit higher plasma progesterone levels at proestrus relative to recipients of high levels of maternal care (High LG). Progesterone has been shown to decrease depressive-like behaviors on the forced swim test. Attenuation of depressive-like behavior may occur through progesterone's upregulation of brain derived neurotrophic factor (BDNF), a neurotrophin that has been negatively correlated with depression symptomatology. We sought to assess whether natural variations in maternal care could induce differences in depressive-like behaviors, and to determine if this effect was mediated by differences in progesterone activity. To obtain High and Low LG offspring for testing, maternal behavior of Long Evans dams was scored on postnatal days 1-6 during five daily observations that occurred every three minutes during 72-min periods. Licking/grooming (LG) behavior 1SD above the cohort mean was characterized as High LG behavior, whereas behavior 1SD below the mean was considered Low LG. Adult female offspring were subjected across the estrous cycle to either a saccharin preference task or a forced swim test. For the saccharin preference task, animals were deprived of water for two hours, then administered a two-bottle choice test for one hour across a 10-day period. For the forced swim test, animals were tested over two days across proestrus and estrus

or estrus and metestrus. Our data show that Low LG offspring preferred saccharin more than Highs, with significant differences at both estrus and diestrus. Low LG females tested on the forced swim task during proestrus and estrus spent the least amount of time floating and had a longer latency to float on the second day of testing. Given the established differences in progesterone levels between Low and High LG female offspring, it is possible that these group differences in behaviors are mediated by this sex hormone. Plasma progesterone levels will be assessed and BDNF protein levels will be quantified in brain regions associated with rodent models of depression.

Disclosures: A. Borrow: None. **M. Stolow:** None. **N.M. Cameron:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.29/GG15

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R21 MH090392

Title: Sex differences in topographically-specific effects of social stress on ventral tegmental area dopaminergic neurons

Authors: *G. GREENBERG[1], M. Q. STEINMAN[2], K. R. SCROGGINS[2], I. E. DOIG[2], D. G. ESQUIVEL[2], B. C. TRAINOR[1];
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Abstract: Activity of mesolimibic dopaminergic neurons in the ventral tegmental area (VTA) has generally been associated with hedonic experiences, but VTA activity has also been linked to aversive stimuli. Indeed, there is increasing evidence for topographical organization of dopaminergic neurons in the VTA, with distinct projections responding to hedonic or aversive stimuli. Social defeat stress is an ethologically valid paradigm for modeling stress in rodents, and it consistently produces a distinct suite of depressive-like symptoms, including social withdrawal. Previous studies in male mice suggest differences in susceptibility to social defeat stress stem from changes in VTA dopaminergic neuron excitability. Whether this system influences differences in female responses to social stress is not known. This is in part because the majority of studies using social defeat in animal models have focused on male rodents. We previously reported sex differences in the effects of social defeat on social withdrawal behavior in California mice (Peromyscus californicus). Here, we randomly assigned male and female California mice to three episodes of social defeat or control conditions. We characterized

neuronal activity within the dorsal-ventral extent of the VTA, employing cFos and tyrosine hydroxylase (TH) double-label immunohistochemistry to indirectly measure dopaminergic neuron activity. Stressed female mice had a significantly greater percent of TH cells in ventral VTA coexpressing cFos when compared to control females, suggesting increased dopaminergic activity in this region. Additionally, stressed female mice had significantly more TH-positive cells in ventral VTA compared to control females. These increases were not observed in stressed male mice. Interestingly, both control male and female mice had significantly increased percent colocalizations in the middle VTA when compared to stressed mice. No effects of stress on percent colocalization were observed in dorsal VTA. These data support the hypothesis that responses of VTA dopamine neurons to aversive contexts are topographically organized.

Disclosures: G. Greenberg: None. **M.Q. Steinman:** None. **K.R. Scroggins:** None. **I.E. Doig:** None. **D.G. Esquivel:** None. **B.C. Trainor:** None.

Poster

542. **Mood Disorders: Animal Models III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 542.30/GG16

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Angiotensin-(1-7) central administration induces anxiolytic-like effects in elevated plus maze and decreased oxidative stress in the amygdala

Authors: *C. ALIN STELIAN[1], W. BILD[2];
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Abstract: There is increasing evidence that besides the well-known angiotensin (Ang) II, other renin-angiotensin system (RAS) peptides, including Ang-(1-7), could have important effects at the central level.
However, very few things are known about the central actions of Ang-(1-7), while the effects of its administration alone on anxiety were not tested to this date, according to our knowledge. In this way, we were interested in studying the effects of Ang-(1-7) intracerebroventricular administration on anxiety levels, as studied through some main behavioral parameters in the elevated plus maze, as well as the importance of Ang-(1-7) in the oxidative stress status from the amygdala, which is one of the key brain regions involved in mediating anxiety.
We report here a possible anxiolytic-like effect of Ang-(1-7) administration, as demonstrated by the increased percentage of time spent and frequency of entries in the open arms of the elevated plus maze, as well as increased head-dipping behavior in the open arms and decreased stretching

in closed arms. Also some antioxidant effects of Ang-(1-7) are suggested since a significant increase of GPX specific activity and a decrease of the main peroxidation marker MDA were observed in the amygdala. Moreover, we found a significant correlation between most of the behavioral parameters in the elevated plus maze and the levels of the oxidative stress markers. However, further studies are necessary in order to elucidate the effects of Ang-(1-7) administration on anxiety and oxidative stress status and also on the possible correlation that might exists between these aspects.

Disclosures: C. Alin Stelian: None. **W. Bild:** None.**Poster**

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.01/GG17

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIDA Grant DA024760

WSU-SOM Department of Psychiatry & Behavioral Neurosciences

WSU-SOM Office of the Vice President for Research

WSU-SOM Department of Neurosurgery

John D. Dingell VA Medical Center

Title: Single prolonged stress: Validity of translating PTSD model into mice

Authors: *A. L. EAGLE[1], K. MULO[1], R. J. KOHLER[1], A. CONTI[2,3], S. A. PERRINE[1]; [1]Psychiatry & Behavioral Neurosci., [2]Neurosurg., Wayne State Univ. Sch. of Med., Detroit, MI; [3]John D. Dingell VA Med. Ctr., Detroit, MI

Abstract: The single prolonged stress (SPS) rat model of posttraumatic stress disorder (PTSD) has shown great potential in understanding the behavioral phenotypes, such as re-experiencing memories triggered by cues associated with the trauma, as well as their underlying neurobiological bases, such as enhanced dexamethasone suppression of the HPA-axis stress response. The current project aimed to translate the SPS model for use in mice and investigate its validity as a rodent model of PTSD. Male C57 mice were exposed to a modified “mouse SPS” (mSPS) paradigm or control treatment and were tested 7 days post stressors. Separate groups of mice were tested for trauma cue-induced freezing, locomotor activity, and anxiety-like (thigmotaxis) behavior in a novel open field or dexamethasone suppression of plasma corticosterone (CORT) levels. For trauma cue-induced freezing a pulsed tone sequence was

paired with the mSPS stressors. Seven days later the mice were placed into a novel open field and reintroduced to the tone. Another group of mice were treated with daily oral paroxetine (10 mg/kg/day), a selective serotonin reuptake inhibitor (SSRI), in their drinking water during the 7 day period after mSPS and then tested for trauma cue-induced freezing behavior. The mSPS treatment increased trauma cue-induced freezing, but when the tone was not present did not produce freezing. The increase in trauma cue-induced freezing was blocked by paroxetine. mSPS also enhanced dexamethasone-induced suppression of plasma CORT. Hippocampal glucocorticoid receptor expression, linked to the enhancement of dexamethasone suppression, is currently being investigated in this model. mSPS did not produce significant changes in either locomotor activity or anxiety-like behavior, mirroring previous findings with SPS in rats. The enhanced trauma cue-induced freezing and increased suppression of plasma CORT reflect clinical symptoms. Blockade of trauma cue-induced freezing by paroxetine, an SSRI and primary treatment of clinical PTSD, models current drug therapy to reduce the symptoms of PTSD. These findings provide strong construct and predictive validity for mSPS as a model of PTSD in mice.

Disclosures: A.L. Eagle: None. **K. Mulo:** None. **R.J. Kohler:** None. **A. Conti:** None. **S.A. Perrine:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.02/GG18

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: RO1-MH068283

Title: The rewarding effects of exercise do not depend on wheel running controllability

Authors: *J. J. HERRERA[1], P. J. CLARK[2], S. M. ENGEL[2], B. N. GREENWOOD[2], M. FLESHNER[2];
[2]Integrative Physiol., [1]Univ. of Colorado, Boulder, CO

Abstract: The mesolimbic reward pathway is implicated in the development and treatment of stress-related psychiatric disorders such as anxiety and depression. Exercise can reduce the incidence of stress-related disorders, but the contribution of exercise reward to exercise-induced stress resistance is unknown. We have reported that the anxiolytic and antidepressant-like effects of exercise are independent of exercise controllability; whereby both voluntary and forced wheel running protect rats against behavioral consequences of stress. Voluntary exercise is a natural reward, but whether rats can find forced wheel running rewarding is unknown. The goal of the

current studies was to determine whether the rewarding effects of wheel running depend on its controllability. Following 1 week of voluntary running, male F344 rats (8 / group) were divided into voluntary and forced groups. The wheels belonging to rats in the forced group were rotated by motors following a predetermined schedule designed to closely resemble the typical pattern of voluntary running behavior. For 5 weeks, rats were placed into their assigned voluntary or forced wheels or, on alternating nights, into an empty cage. Two hours after wheel or empty cage exposure, rats were placed on one distinct side of a conditioned place preference (CPP) chamber. One side was always paired with running (paired) and the opposite side was paired with the empty cage (unpaired). Probe tests to determine CPP were conducted 1, 3, and 5 weeks after the start of CPP training. After the final probe trial, and 24 hours after the last running exposure, rats were placed on either the paired or unpaired side and sacrificed 30 minutes later. Double in situ hybridization (FISH) was used to assess potential conditioned activation of reward circuitry elicited by acute exposure to the side paired with exercise. Finally, pCREB expression in the medial and lateral VTA, subregions of the VTA implicated in the signaling of reward and aversion respectively, were quantified in a separate cohort of rats given access to voluntary or forced wheels for 6 weeks. Results suggest that both voluntary and forced wheel running are rewarding. Rats spent more time on the side of the CPP chamber paired with exercise during each probe trail, regardless of wheel running controllability. Moreover, both voluntary and forced wheel running increased pCREB in DA neurons in the medial, but not lateral, VTA. Analyses of the double FISH are currently underway. Together, these data suggest that both voluntary and forced wheel running can be rewarding. The rewarding effects of exercise could contribute to the mechanisms by which exercise increases stress resistance.

Disclosures: J.J. Herrera: None. **P.J. Clark:** None. **S.M. Engel:** None. **B.N. Greenwood:** None. **M. Fleshner:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.03/HH1

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH068283

DARPA W911NF-10-1-0050

Title: The impact of voluntary exercise on stress-induced disruptions in diurnal rhythms of sleep and physiology

Authors: *R. S. THOMPSON[1,2], B. N. GREENWOOD[1,2], M. FLESHNER[1,2];
[1]Univ. Colorado Boul, BOULDER, CO; [2]Ctr. for Neurosci., Univ. of Colorado at Boulder, Boulder, CO

Abstract: Exercise can increase resistance to stress-related psychiatric disorders such as anxiety and depression. One way exercise may confer stress resistance is by reducing the impact of stress on sleep and diurnal physiological rhythms; disruptions of which are thought to contribute to stress-related disorders. Indeed, exercise is a powerful non-photic entrainment cue to the biological clock and thus could help prevent or reverse stress-induced disruptions in diurnal rhythms. Adult male F344 rats (8 per group) remained sedentary or had voluntary access to wheels for 6 weeks. After 4 weeks of exercise, rats were implanted with F40-EET biotelemetry devices (DSI) and real-time continuous diurnal/circadian rhythms were recorded. Following a 1-week recovery period, rats were again allowed access to running wheels for an additional 2 weeks. Diurnal rhythms of locomotor activity, heart rate, body temperature, and sleep (i.e. REM, NREM, and WAKE) were continually recorded in the presence of a 12hr light/dark cycle in running and sedentary rats. After a total of 6 weeks of exercise, both the sedentary and running rats were exposed to a single acute stressor (100, 5-s, 60-s ITI inescapable tail shock) previously reported to disrupt physiological diurnal rhythms and produce behaviors resembling symptoms of affective dysregulation. Physiological parameters were measured prior to, during, and following stressor exposure. Compared to sedentary rats, exercise rats had larger diurnal amplitudes of locomotor activity rhythms, core body temperatures and %REM during the light (inactive) cycle prior to stressor exposure. The increase in REM persisted following stress in the exercise rats. During stress, both groups reached maximal heart rate, but physically active rats reached a higher maximum body temperature. Stressor exposure impacted diurnal rhythms of activity, heart rate, body temperature, and sleep in both sedentary and exercise rats; and these changes persisted for 48-96 hours. These data suggest that protection against stress-induced disruptions in diurnal rhythms and increases in REM sleep could contribute to the cognitive and affective benefits of exercise.

Disclosures: R.S. Thompson: None. **B.N. Greenwood:** None. **M. Fleshner:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.04/HH2

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Dutch Ministry of Defense

Title: Plasma testosterone levels and the development of posttraumatic stress symptoms after military deployment: A prospective cohort study

Authors: A. REIJNEN, *E. GEUZE, E. VERMETTEN;
Military Mental Hlth/UMC, Utrecht, Netherlands

Abstract: Introduction Testosterone is a steroid hormone that plays a role in the regulation of sexuality, aggression, cognition and emotions. Several studies suggest that plasma testosterone levels are altered after acute physical and psychological stress. As of yet it is unknown whether testosterone levels are altered following prolonged exposure to high-intensity stress. Military deployment is a period of prolonged stress in which military personnel is often exposed to stressful experiences. The aim of the present study was to investigate the effect of military deployment on testosterone levels. In addition, the predictive value of testosterone levels on the development of posttraumatic stress symptoms was assessed.
Method This study is part of a prospective cohort study on deployment-related health problems in the Dutch armed forces. Morning plasma testosterone levels of 481 male soldiers were assessed before and 1 month and 6 months after deployment to Afghanistan. Participants were assigned to the PTSD or comparison group based on their scores on the Self-Rating Inventory for PTSD symptoms after deployment.
Results Preliminary results indicate that testosterone levels before and after deployment were not significantly different. In addition, there were no statistically significant differences in plasma testosterone levels before and after deployment between the participants with a high level of PTSD symptoms and the comparison group. However, the level of testosterone directly after deployment was found to predict the development of a high level of PTSD symptoms in the two years after deployment.
Discussion The finding that plasma testosterone levels are comparable between the group with a high level of PTSD symptoms and the comparison group is in agreement with other studies. The current study also showed that testosterone levels after deployment did not differ from the baseline measures. These results suggest that, whereas alterations were found in acute stress situations, the hypothalamic-pituitary-gonadal (HPG) system might adapt under prolonged psychological stress.

Disclosures: A. Reijnen: None. **E. Geuze:** None. **E. Vermetten:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.05/HH3

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH068283

DARPA W911NF-10-1-0050

Title: Identifying mechanisms by which exercise prevents inescapable stress induced instrumental learning deficits

Authors: *P. J. CLARK[1], J. AMAT[2], P. R. GHASEM[3], S. O. MCCONNELL[3], S. F. MAIER[2], B. N. GREENWOOD[3], M. FLESHNER[3];
[2]Psychology & Neurosci., [3]Integrative Physiol., [1]Univ. of Colorado-Boulder, Boulder, CO

Abstract: The World Health Organization predicts depression will become the largest economic health burden on society by 2030. However, the neural mechanisms that underlie depression are not well understood. Exposure to uncontrollable stress is a major risk factor for developing mood disorders like depression. In rat models, exposure to inescapable stress (IS) produces a sequelae of depression-like behaviors including deficits in the shuttle box escape task, a form of instrumental learning. Shuttle box escape deficits following IS are dependent on sensitized serotonin (5-HT) activity at receptors in the dorsal striatum during the mild stress (foot shock) used to motivate task acquisition. Our lab has observed that shuttle box escape deficits following IS are prevented in rats that engage in voluntary wheel running for 6 wks prior to IS. Yet, comparatively less is known about how exercise prevents such deficits. One hypothesis is that exercise attenuates IS-sensitized 5-HT transmission in the dorsal striatum during mild stress, which may consequently impact activity of additional neurotransmitters critical for instrumental learning including dopamine (DA). Attenuated 5-HT and/or potentiated DA transmission in the striatum of running animals during stress may increase activity in populations of striatal neurons hypothesized to signal reward or reduce activity in neurons involved in aversion, which could provide protection against shuttle box deficits. The purpose of this experiment was two-fold. 1) Identify the effects of mild stress (two foot shocks, 0.8mA, 5s duration) applied 24h following IS on extracellular concentrations of 5-HT and DA in the dorsal medial and lateral striatum of running and sedentary adult male Fischer 344 rats using *in vivo* microdialysis. 2) Identify whether 6 wks of running in Fischer 344 rats alters neural activity (as measured by *c-fos*) elicited by IS in populations of dynorphin (reward signaling) and enkephalin (aversion signaling) expressing dorsal striatal medium spiny neurons using fluorescent *in situ* hybridization. Results indicate that IS-induced sensitization of extracellular 5-HT is blunted, and DA concentrations are potentiated in the dorsal medial striatum following mild stress of running compared to sedentary rats. Data collection for
the dorsal lateral striatum is currently underway. Moreover, preliminary results suggest 6 wks of running decreases the number of enkephalin neurons expressing the activity marker *c-fos* during IS compared to

sedentary rats. Taken together, these data provide a potential mechanism by which 6 wks of exercise prevents depression-like deficits in instrumental learning following IS.

Disclosures: P.J. Clark: None. **J. Amat:** None. **P.R. Ghasem:** None. **S.O. McConnell:** None. **S.F. Maier:** None. **B.N. Greenwood:** None. **M. Fleshner:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.06/HH4

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: BID-PICT-2011-1015

PIP 02546

IBRO-SfN Travel Grants 2013

Title: Reduced hypothalamic NOS activity and CB1 mRNA cannabinoid receptors are related to behavioral impairments in stressed rats

Authors: B. BURDET[1], D. G. MAUR[1], A. DE LAURENTIIS[1], V. RETTORI[1], *M. ZORRILLA ZUBILETE[2,1];
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Abstract: Chronic stress leads to activation of the hypothalamo-pituitary-adrenal axis (HPA) and changes in different parameters in limbic areas associated with stress. The hypothalamus is the main brain structure involved in the neuroendocrine control of stress and anxiety and it has been demonstrated that nitric oxide (NO), a free radical produced by nitric oxide syntase (NOS), participates in these processes. On the other hand, there is evidence that endocannabinoid system can modulate stress responses. The aim of the present work was to study the effect of chronic restraint stress on behavioral impairments and the participation of the endocannabinoid system and NO in these effects (using a NOS inhibitor, L-NAME and a NO donor, Molsidomine). Restraint stress was applied to adult Sprague-Dawley male rats for two hours daily during 7 consecutive days (7d). Inhibitory avoidance and elevated plus maze tests, NOS activity, oxytocin and corticosterone plasma levels and cannabinoid receptor type 1 (CB1) levels in hypothalamus were evaluated. A significant decrease ($p<0.001$) in the activity of hypothalamic total NOS was found in stressed animals respect to control (C) animals. The expression of hypothalamic CB1

mRNA was significantly reduced ($p<0.05$) after restraint stress. Oxytocin and corticosterone plasma levels were significantly increased in stressed rats ($p<0.01$ and $p<0.05$ respectively). Moreover, we found a significant decrease in the latency to enter into the dark compartment in the 7d respect to C ($p<0.05$) as well as in 7d+Veh and C+L-NAME groups respect to C+Veh ($p<0.01$ and $p<0.05$ respectively) in the inhibitory avoidance test. Also, a significant increase in the percentage of entries and time spent in the open arms in the 7d respect to C ($p<0.05$) as well as in 7d+Veh and C+L-NAME groups respect to C+Veh ($p<0.05$) was found in the elevated plus maze test. In conclusion, our model of stress elicited anxiolytic-like behavior and a deficit in associative memory that could be related to the decrease in NO production and CB1 receptors levels in the hypothalamus. Also the increase in oxytocin plasma levels could contribute to the behavioral changes observed.

Disclosures: B. Burdet: None. **D.G. Maur:** None. **A. De Laurentiis:** None. **V. Rettori:** None. **M. Zorrilla Zubilete:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.07/HH5

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: JSPS KAKENHI Grant Number 24 1833

Title: Central administration of corticotropin-releasing factor receptor antagonist, alpha-helical CRF (9-41), does not alter stress-induced enhancement of conditioned fear response in rats

Authors: *R. RYOKE, K. YAMADA, Y. ICHITANI;
Psychology & Behavioral Neurosci., Univ. of Tsukuba, Tsukuba / Ibaraki, Japan

Abstract: Posttraumatic
Stress Disorder (PTSD) is an anxiety disorder that may develop following to an exposure to a highly traumatic event. Up to today, many researchers have proposed animal models to study the neurobiological mechanisms underlying PTSD. Most of these models have been derived from Pavlovian fear conditioning, and it is known that prior exposure to traumatic stress enhances conditional fear responding to subsequent mild stress. We previously reported that the multiple stress (MS) consisted of foot shocks and forced swimming had long-lasting (more than 2 weeks) effects on subsequent fear conditioning. And, these effects were not influenced by suppression of stress-induced secretion of glucocorticoids (GCs) by metyrapone or adrenalectomy. In this study, we examined whether pharmacologically blocking central CRF receptors would affect the effects

of the MS on subsequent contextual fear conditioning. Two different shock chambers, Context A chamber and Context B chamber, were used for delivering the MS and conducting the fear conditioning, respectively. These chambers differed in the context including illumination, background sound and so on. Male Wistar-Imamichi rats were exposed to the MS consisted of 4 spaced foot shocks (1 mA, 1 s) for 25 min in the Context A chamber and forced swimming for 20 min in a plastic bucket. Non-stressed control animals were placed in the Context A chamber and a plastic cage for the same amount of time as the MS animals without foot shocks and swimming, respectively. Either CRF receptor antagonist, alpha-helical CRF (5 or 20 μg, i.c.v.), or saline were administrated 20 min before exposure to the Context A chamber. Contextual fear conditioning was conducted 14 days after the MS. All animals were exposed 2 mild foot shocks (0.1 mA, 2 s) in the Context B chamber. Conditioned fear response (freezing) in the Contex B chamber was assessed in three retention tests, which were conducted on the next, 7 and 14 days after the conditioning. The results showed that the central administration of alpha-helical CRF did not affect the MS-induced enhancement of conditioned fear response. These results suggest that central activation of CRF receptors triggered by MS is not involved in regulating the long-term stress-induced sensitization of fear.

Disclosures: R. Ryoke: None. **K. Yamada:** None. **Y. Ichitani:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.08/HH6

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH068283

NIH R03 MH050479

Title: Voluntary exercise during fear extinction reduces fear renewal: Role for activation of reward circuitry during extinction

Authors: *C. BOUCHET[1], A. MIKA[2], K. G. SPENCE[2], J. E. HELLWINKEL[2], S. CAMPEAU[3], H. E. W. DAY[3], M. FLESHNER[2], B. N. GREENWOOD[2];
[2]Integrative Physiol., [3]Psychology and Neurosci., [1]Univ. of Colorado, Boulder, CO

Abstract: Recent efforts to treat anxiety and fear disorders focus on fear extinction. Exposure therapy in humans has limited efficacy, however, because fear memories often resurface when the extinguished conditioned stimulus (CS) is presented in a new context (fear renewal). Emerging evidence suggests that extinction involves both cognitive and affective processes. Cognitive extinction processes could support learning of a new CS-NoUS association; whereas affective processes could support the learning of a new (less aversive) emotional "state" assigned to the CS. Manipulations that target both the cognitive (such as noradrenergic (NE) signaling) and affective (such as activation of striatal reward circuits) extinction processes could strengthen extinction memories and make them resistant to contextual modulation during renewal. Acute exercise is both arousing (elevates NE) and rewarding (activates striatal reward circuits); thus could potentially act as an adjunct therapy during extinction to reduce fear renewal. To address this possibility, male F344 rats without (Locked) or with (Run) 3 nights of experience with running wheels, were conditioned to fear a tone CS. Fear extinction (15 CS presentations) took place the next day in the familiar, locked or mobile running wheels. Run rats ran during extinction, and this running was associated with a reduction in freezing and corticosterone during renewal tested in a novel context 1 week later. Run and Locked rats extinguished in a control context displayed identical levels of fear during renewal. Administration of naloxone (5.0 mg/kg; to block exercise reward) immediately prior to extinction reduced the effect of running during extinction on renewal tested 1 week later. These data suggest that exercise during extinction, but not a history of exercise per se, reduces renewal of extinguished fear through a mechanism involving exercise reward. Reward during extinction could reduce renewal by facilitating the learning of a new, less aversive, CS "state;" perhaps via second order conditioning. Consistent with this possibility, double fluorescent in situ hybridization revealed that CS exposure during renewal elicited greater activity (c-fos+) of the reward-associated direct pathway and an attenuation of activity of the aversion-associated indirect pathway of the striatum in rats that ran during extinction, relative to Locked rats. These data suggest that recruiting an affective extinction process could result in an extinction memory that is less susceptible to contextual modulation. Studies are underway to determine if CS value "state" can be coded by relative activation of direct and indirect striatal pathways.

Disclosures: C. Bouchet: None. **A. Mika:** None. **K.G. Spence:** None. **J.E. Hellwinkel:** None. **S. Campeau:** None. **H.E.W. Day:** None. **M. Fleshner:** None. **B.N. Greenwood:** None.

Poster

543. Behavioral Effects of Stress

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.09/HH7

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH063344

ASPIRE II Funding- Univ South Carolina

Title: Modeling individual differences in behavioral stress responses to uncover neurobiological mechanisms for resiliency to PTSD

Authors: *M. A. WILSON, A. C. SHARKO, K. F. KAIGLER, A. HAND, A. KERSNOWSKI, M. P. KELLY, J. R. FADEL;
Dept Pharmacol,Physiol,Neurosci, Univ. South Carolina, Sch. Med., COLUMBIA, SC

Abstract: Post-traumatic stress disorder (PTSD) is a severe anxiety disorder that can develop after experiencing a life-threatening trauma, such as combat service, assault, or a natural disaster. However, not everyone who experiences these types of traumas develops PTSD, suggesting that some neurobiological factors may confer resiliency, or risk, to the long-term negative effects of traumatic stressors. Research into the neurobiological mechanisms that underlie resiliency has been limited by a lack of animal models that naturally show differential sensitivity to traumatic stress. Previously, our laboratory has demonstrated that outbred Long-Evans rats show individual differences in unconditioned anxiety-like behavior, as measured on the elevated plus maze. To determine if this rat strain also shows individual differences in conditioned anxiety-like behavior, we tested several groups of rats using a conditioned freezing paradigm with or without a prior exposure to predator (ferret) odor as a traumatic stress. Conditioned freezing is useful for modeling the symptoms of re-experiencing trauma seen in patients with PTSD. One week after exposure to control or predator odor, we examined differences in acquisition of conditioned freezing, contextually conditioned freezing, and cue conditioned freezing, as well as latency to extinguish cue conditioned freezing behaviors over several trials of extinction learning. In several different cadres of animals, approximately one third of each group of 12-14 rats failed to completely extinguish the conditioned freezing behavior, even after 20 presentations of the conditioned tone cue, and also demonstrated poor retention of extinction learning 48 hrs and 1 week later. Individual differences between these groups were also seen in subsequent startle and risk-assessment behaviors, as well as neuronal activation in the prefrontal cortex using cFos immunohistochemistry in response to predator odor exposure. These results correlate with clinical statistics showing that approximately 30% of individuals who experience a traumatic event eventually develop PTSD. Additional studies are examining individual differences in patterns of neuronal activation in the prefrontal
cortex and extended amygdala associated with these behavioral patterns.

Disclosures: M.A. Wilson: None. **A.C. Sharko:** None. **K.F. Kaigler:** None. **A. Hand:** None. **A. Kersnowski:** None. **M.P. Kelly:** None. **J.R. Fadel:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.10/HH8

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NDSU Research Grant

Title: Evaluating the stress-reducing potential of coffee volatiles in socially isolated mice using stress-induced hyperthermia and open-field tests

Authors: *Y. HAYASHI[1], S. SOGABE[1], M. SUZUKI[1], J. TANAKA[2];
[1]Notre Dame Seishin Univ., Okayama, Japan; [2]Naruto Univ. of Educ., Naruto, Japan

Abstract: We previously reported the stress-reducing potential of coffee volatiles in social mice using the elevated plus maze, pentobarbital-induced sleep and stress-induced hyperthermia tests. Social isolation induces anxiety, depression, and aggression in laboratory animals. Thus, socially-isolated mice appear to be a more suitable experimental model to study putative anxiolytic-like effects of chemical compounds. To further clarify the relationship between coffee volatiles and psychological stress, this study investigated the stress-reducing effects of coffee volatiles in socially-isolated mice using stress-induced hyperthermia and open field tests. Male ICR mice aged 4 weeks and weighing 18-23 g were used. Animals were kept either in social isolation (1 mouse per cage) or in social (5 mice per cage) groups for 10 weeks or more prior to testing. Fresh medium-dark roasted and moderately powdered Guatemalan coffee beans were used. The coffee powder was placed into several plastic cases in a test room with the temperature maintained at 25°C ± 1°C. Mice were exposed to coffee volatiles in the test room. Four hours after the beginning of the exposure period, stress-induced hyperthermia and open field tests were performed. In the stress-induced hyperthermia test, in the absence of coffee volatiles, body temperature elevation was significantly higher in the socially isolated group than in the social group. After four hours of coffee volatile exposure, body temperature elevation in the socially-isolated group was reduced and was similar to that of the social group. In the open field test, without coffee volatiles, defecation was increased in socially isolated mice; other parameters were the same as in the social group. Coffee volatiles decreased defecation in socially-isolated mice. These results suggest that volatile compounds in roasted coffee beans have stress-reducing effects in socially-isolated mice as well as in social mice.

Disclosures: Y. Hayashi: None. **S. Sogabe:** None. **M. Suzuki:** None. **J. Tanaka:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.11/HH9

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH068283

Title: Wheel running produces widespread structural alterations within striatal and limbic regions involved in stress

Authors: *P. R. GHASEM[1], A. MIKA[2], E. A. SISNEROS[2], M. A. KEAG[2], S. M. ENGEL[2], P. J. CLARK[2], B. N. GREENWOOD[2], M. FLESHNER[2];
[1]Univ. of Colorado At Boulder, Boulder, CO; [2]Integrative Physiol., Univ. of Colorado at Boulder, Boulder, CO

Abstract: Exercise increases resistance against stress-related disorders such as depression and anxiety, but the mechanisms remain unknown. Exercise produces neuro-plastic and functional adaptations within a variety of brain regions implicated in the behavioral consequences of stress. In the hippocampus, for example, wheel running produces increases in dendritic complexity that parallel improvements in contextual and spatial memory. The effects of exercise on neuronal structure within other brain regions affected by stress, however, remain unexplored. Among the behaviors impacted by stress that are prevented by prior exercise are anxiety-like behaviors (putatively involving the amygdala) and deficits in instrumental learning (putatively involving the dorsal striatum). We examined, therefore, the effects of voluntary wheel running on dendritic arborization in the amygdala and striatum. Adult, male Fisher (F344) rats (n=7/grp for hippocampal analysis and n=8/grp for striatum/amygdala analyses) were allowed either voluntary access to running wheels or remained sedentary for 6 weeks. Brains were immediately removed and processed in accordance with Golgi Stain protocols. Sholl analysis was used to quantify pyramidal cells with the CA3 region of the hippocampus and medium spiny neurons within the dorsal striatum. Wheel running increased dendritic complexity within the CA3 region of the hippocampus as well as within the dorsal striatum. Amygdala analyses are in progress. These data suggest that exercise produces extensive alterations in neuronal structure that may be involved in stress resistance.

Disclosures: P.R. Ghasem: None. **A. Mika:** None. **E.A. Sisneros:** None. **M.A. Keag:** None. **S.M. Engel:** None. **P.J. Clark:** None. **B.N. Greenwood:** None. **M. Fleshner:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.12/HH10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: VA Merit Award 1I01BX001075

Title: Impact of chronic variable versus acute stress experience on conditioned fear memory and sensitized acoustic startle responses in rats

Authors: S. SCHMELTZER[1,3], L. VOLLMER[2,1], C. DOULGAS[2,3], M. WEINERT[2], J. RUSH[2], R. SAH[2,1,3];
[1]Neurosci. Grad. Program, [2]Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH; [3]VA Med. Ctr., Cincinnati, OH

Abstract: Exposure to intense traumatic events on an acute or chronic scale can lead to posttraumatic stress disorder (PTSD). Recent studies have demonstrated a steep dose-response curve between trauma frequency and PTSD symptom severity, such that the more traumatic events a person experiences, the greater the intensity of PTSD symptoms. Although this observation has been around in the clinic, there is little evidence on underlying mechanisms. To investigate this phenomenon, rats were exposed to chronic, unpredictable aversive experiences that were superimposed with a primary or "index traumatic event" in the form of electric shocks (chronic variable stress-shock; CVS-S). The comparison group was an acute shock (AS) group which was exposed only to the index traumatic event (shocks). We hypothesized that the CVS-S group would have worse behavioral outcomes. To test this, we measured conditioned fear responses (freezing) and acoustic startle response, which measures sensitized responses. We also assessed neuronal activation using delta fosB and cFos immunoreactivity in fear regulatory brain areas. Consistent with our hypothesis, fear acquisition, recall, and reinstatement were significantly potentiated in the CVS-S rats. No differences were noted in peak startle responses; however CVS-S rats showed improved habituation to startle. These behavioral changes were accompanied by increased neuronal activity in the infralimbic prefrontal cortex and basolateral amygdala of CVS-S rats. In conclusion, our data suggest that chronicity and magnitude of stress or traumatic events worsens fear memory related conditioned responses but may facilitate adaptation to novel aversive encounters.

Disclosures: S. Schmeltzer: None. **L. Vollmer:** None. **C. Doulgas:** None. **M. Weinert:** None. **J. Rush:** None. **R. Sah:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.13/HH11

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Role of kynurenic acid in regulating neurochemical and behavioral responses to stress

Authors: *J. I. KOENIG[1], H.-Q. WU[1], T. FUKUWATARI[2], R. SCHWARCZ[1];
[1]Univ. Maryland Sch. Med., BALTIMORE, MD; [2]Food Sci. and Nutr., The Univ. of Shiga Prefecture, Hikone, Shiga, Japan

Abstract: Kynurenic acid (KYNA), one of the principal products of the kynurenine pathway of tryptophan degradation, is an endogenous antagonist of both the NMDA and the alpha-7 nicotinic receptor. In addition, KYNA is an important modulator of forebrain dopaminergic neurons. Stress exposure also modulates dopamine (DA) mechanisms and increases the release of DA in forebrain terminal regions, such as the prefrontal cortex (PFC). Glucocorticoid hormones (GC) appear to mediate stress-induced activation of the ascending DA neurons. These actions of GC on DA may be important in the etiology of depressive behaviors. However, there are also clinical situations when GC responses to stress are blunted, such as in PTSD, and our understanding of the consequences of an inadequate GC response to stress is limited. To test the possible role of KYNA - and specifically of KYNA-DA interactions - in this context, we studied neurochemical and behavioral responses to acute stress in adrenalectomized (ADX) rats. Adult, male animals were surgically ADX or sham operated and received saline to drink after surgery. Six days later, microdialysis guide cannulae were implanted over the PFC. The next morning, microdialysis probes were inserted, and perfusate samples were collected during a 2-hour baseline period, a 2-hour restraint stress and a 4-hour recovery period. Extracellular levels of KYNA and DA were then determined in the same microdialysate by HPLC. In sham rats, restraint stress elevated PFC DA while having no effect on KYNA. In stark contrast, in GC-insufficient ADX rats, DA levels were unaffected by stress exposure while KYNA levels were significantly elevated. Acute pretreatment of ADX rats with a selective, orally active inhibitor of KAT II (the enzyme responsible for KYNA synthesis), restored the DA elevation in response to restraint stress. Using a behavioral outcome measure (fear extinction), we found that ADX rats lacked the cognitive flexibility to extinguish freezing behavior in a long-term memory test. However, ADX rats readily learned the association between tone cues and foot shocks during conditioning trials, and displayed intact short-term memory for the pairing. Acute KAT II inhibition normalized fear extinction in ADX rats. Together, these studies reveal that removal of GC tone disrupts the dynamic regulation of DA mechanisms by endogenous KYNA in the PFC, leading to an inability to extinguish fear memories. Our results suggest that pharmacological down-regulation of KYNA formation may provide a fundamentally novel approach to thwart

dysfunctional memory extinction. This approach may be useful in the treatment of PTSD and other stress-related clinical conditions.

Disclosures: J.I. Koenig: None. **H. Wu:** None. **T. Fukuwatari:** None. **R. Schwarcz:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.14/HH12

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: DFG Grant HE 1128/16 - 1 and

DFG Grant RI 1922/1 - 1

Title: Stress-induced changes in 5-HT receptor-mediated neuromodulation of granule cell inhibition in a rat model of PTSD

Authors: D. GRUBER[1], *K. E. GILLING[1], A. ALBRECHT[2,3], O. STORK[3], G. RICHTER-LEVIN[2], U. HEINEMANN[1], J. BEHR[1,4];
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Abstract: Abstract
Substantial evidence indicates that after stressful life events, a high anxiety trait may develop into posttraumatic stress disorder (PTSD) or depression. By activation of the median and dorsal raphe nuclei, which send a major serotonergic input to the hippocampus, stressful events enhance serotonin levels in the dentate gyrus, a region highly susceptible to stress. Using an anxiety disorder model (Tsoory and Richter-Levin 2006), we assessed the effects of stress encountered in juvenility, adulthood and the combination thereof upon behaviour and, using whole cell recordings, examined the 5-HT-mediated modulation of cellular properties and synaptic inhibition of ventral dentate gyrus granule cells. Animals stressed as adults showed depressive-like behaviour in the open field test only after having been exposed to a stressful challenge in juvenility. In controls, serotonin hyperpolarized cells, decreased their input resistance and reduced the amplitude of evoked IPSCs via the 5-HT_{1A} receptor subtype. The latter effect was significantly reduced in animals stressed in juvenility or in adulthood but not in animals stressed

at both ages. Moreover, we found that stress applied in juvenility or in adulthood reduced miniature IPSCs (mIPSCs) and 5-HT-induced bursts of mIPSCs mediated by presynaptic 5-HT_3 receptors. In animals stressed both in juvenility and in adulthood, the reduction in mIPSCs and 5-HT-induced bursts of mIPSCs was significantly smaller. We hypothesize this effect might be due to a reduction in RNA expression levels of 5-HT_3 receptor transcripts found in singly stressed animals, an effect not seen in animals stressed twice. We conclude that in animals exposed to combined stress, the stressful reminder cue given in adulthood results in depressive-like behavior of the animals that is not accompanied with changes of the 5-HT_{1A} and 5-HT_3-induced modulation of synaptic inhibition. In contrast, the exposure of animals to a single stressful experience does not result in behavioral changes, but causes a decrease of 5-HT_{1A}-induced disinhibition and 5-HT_3-induced bursts of mIPSCs. We suggest that stress-induced changes of the serotonergic system and its impact on hippocampal inhibition may provide a protective molecular mechanism that prevents the expression of depressive-like behavior.

Disclosures: D. Gruber: None. **K.E. Gilling:** None. **A. Albrecht:** None. **U. Heinemann:** None. **J. Behr:** None. **G. Richter-Levin:** None. **O. Stork:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.15/HH13

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: VA ORD RRDD7020R

NIMH R01MH091872

NIMH R01MH087692

Title: Aggression in social contests in veterans with post-traumatic stress disorder

Authors: *L. ZHU[1,2], C. ROSOFF[1,2], K. MCCURRY[2,2], C. B. FRUEH[3], P. H. CHIU[1,2,4], B. KING-CASAS[1,2,4];
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Abstract: Competitive social interactions involve detecting opponents' actions, extracting relevant information from those actions, and selecting behavioral reactions accordingly.

Dysfunction in receiving or processing behavioral signals from opponents during social contests may result in aberrant emotion regulation, aggression and violence. We tested this hypothesis by studying a large sample of veterans with combat-related post-traumatic stress disorder (PTSD), a group of individuals characterized by hyper-arousal symptoms such as irritability and outbursts of anger.
162 OEF/OIF/OND veterans were recruited in the metro Houston, TX and Southwestern Virginia areas. Participants included 103 veterans meeting criteria for current PTSD. We combined functional neuroimaging and computational modeling with a multi-round interpersonal resource competition that has successfully elicited social information learning signals among healthy subjects in the previous neuroimaging study. We formulated a social reinforcement learning model to examine how the brain encodes and computes social learning signals used to guide behavior in subsequent contests among veterans, to pursue the hypothesis that subjects with combat-related PTSD are pathologically perturbed in the processing of social signals related to opponent's actions, and hence demonstrate aberrant aggressive behavior during competitive social interactions.
Preliminary behavioral analysis suggested that the behavioral estimates of individual differences in social learning was significantly correlated with self-reported measures of anger and aggression, combat exposure, and current PTSD symptom status. These results suggest that the proposed computational model captures an important connection between behaviorally revealed aggression and PTSD symptom status, providing insights into the computational process underlying aberrant decisions at the behavioral level. Additionally, our findings point to further investigations into those decisions at a neuromechanistic level.

Disclosures: L. Zhu: None. **C. Rosoff:** None. **K. McCurry:** None. **C.B. Frueh:** None. **P.H. Chiu:** None. **B. King-Casas:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.16/HH14

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH068283

Title: The persistence of exercise-induced stress resistance depends on the developmental stage during which exercise is initiated

Authors: *A. MIKA, C. A. BOUCHET, K. G. SPENCE, B. N. GREENWOOD, M. FLESHNER;
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Abstract: Exercise reduces the incidence of stress-related psychiatric disorders in humans and prevents the development of anxiety- and depressive-like behaviors in rodents, including exaggerated fear and deficits in shuttle box escape learning. When wheel running is initiated around puberty (PND 45-49), the protective effects of exercise persist for 15 days, but are lost by 25 days following forced cessation of exercise. The brain is especially plastic during the prepubertal period, such that the time before puberty could present a window of opportunity for exercise to produce adaptive, stable changes. We therefore examined the effects of exercise initiated in the prepubertal period on the persistence of the stress-protective effects of voluntary wheel running following exercise cessation. Adult (PND 70) and juvenile (PND 24) male, Fisher (F344) rats where allowed access to a running wheel or remained sedentary for 6 weeks. All wheels were then rendered immobile, and rats were exposed to no stress or uncontrollable stress 14 or 24 days later. 24 h following uncontrollable stress, behavioral testing for shock-elicited fear and shuttle box escape occurred, so that exercised rats were forced to remain sedentary for either 15 or 25 days prior to testing. When wheel running was initiated during adulthood (PND 70), the protective effects of exercise on exaggerated fear and shuttle-box escape deficits were lost by 15 days. Interestingly, when wheel running was initiated during the juvenile period (PND 24), the protective effects of exercise persisted longer, and showed no signs of attenuating at 25 days. These results suggest that exercise during early sensitive developmental periods can alter the trajectory of brain development to produce long-lasting stress resistance to the behavioral consequences of exposure to traumatic events during adulthood.

Disclosures: A. Mika: None. **C.A. Bouchet:** None. **K.G. Spence:** None. **B.N. Greenwood:** None. **M. Fleshner:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.17/HH15

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: ERC GenAnx project

Academy of Finland

Title: Behavioral outcome of chronic social defeat in four inbred mouse strains

Authors: E. SOKOLOWSKA[1], S. KÄNGSEP[1], Z. MISIEWICZ[1], V. VOIKAR[2], *I. HOVATTA[1,3];
[2]Neurosci. Ctr., [1]Univ. Helsinki, Helsinki, Finland; [3]Mental Hlth. and substance Abuse Services, Natl. Inst. for Hlth. and Welfare, Helsinki, Finland

Abstract: Social defeat paradigm is a social stress model involving daily physical interaction and sensory contact with an unfamiliar aggressive male. This chronic stress results in behavioral changes including social impairment and increase in anxiety and depression-like behaviors. On a molecular level, these effects are mediated by the brain-derived neurotrophic factor in the nucleus accumbens and hippocampus and the corticotropin-releasing factor in the amygdala. However, also other mechanisms are likely important in these and other brain regions. Different inbred mouse strains show distinct responses to stressful stimuli, implying that genetic factors are important in determining susceptibility and resilience to stress. We subjected animals from four strains (C57BL/6NCrI, BALB/cAnNCrI, DBA/2NCrI, 129S2/SvPasCrI) to social defeat paradigm (10 days) by introducing them briefly into the home cage of an aggressive male (CD1) followed by sensory contact for 24 h. To evaluate consequences of repeated defeat we measured body weight, social preference, locomotor activity, and behavior in open field and light/dark box tests. Repeated defeat affected social preference in all four strains, however a larger number of innately non-anxious C57BL/6 mice were resilient to stress, while most of the innately anxious DBA/2 mice were susceptible (C57BL/6 > BALB/c >129S > DBA/2). Mice that experienced defeat had lower locomotor activity, except the 129S mice, which showed stress-related increase in activity. The defeat procedure did not affect normal increase in body weight, except that weight gain was slower in DBA/2 resilient mice (controls vs. resilient, $p<0.01$ and resilient vs. susceptible, $p<0.05$). Social defeat did not affect behavior in open field or light/dark box tests. Our results demonstrate strain-distinctive response to social defeat stress. Overall, innately anxious DBA/2, 129S, and BALB/c mice were more sensitive to social stress than non-anxious C57BL/6 mice, with different levels of behavioral changes in general locomotor activity and social aversion. The different coping strategies to social stress in different strains are likely partially mediated by genetic factors, reflected in perturbed gene regulatory networks amenable for identification with sequencing-based methods.

Disclosures: E. Sokolowska: None. **S. Kängsep:** None. **Z. Misiewicz:** None. **V. Voikar:** None. **I. Hovatta:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.18/HH16

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Chronic quinpirole administration in rats reduces exploratory behavior and increases water contrafreeloading while preserving responsiveness to changing contingencies

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Abstract: INTRODUCTION
Contrafreeloading, or working for a freely available reward, is thought to be a form of exploratory behavior. In a putative model of compulsive checking, chronic treatment with the D2/D3 dopamine agonist quinpirole increases contrafreeloading for water in rats. In humans, compulsive checking is performed to reduce anxiety, and checking rituals may be altered or abandoned when the environment changes. Whether a similar degree of flexibility exists in drug-induced contrafreeloading remains unclear. Additionally, this effect of quinpirole could be explained by an increase in anxiety or by an elevated exploratory drive.
OBJECTIVES
We assessed the ability of quinpirole-treated rats with contrafreeloading experience to adapt to changing contingencies by requiring them to alternate between response levers for reinforcement. We then examined the effect of quinpirole on open arm exploration in the elevated plus-maze (EPM).
METHODS
In the contrafreeloading study, 12 saline- and 11 quinpirole-treated (0.5 mg/kg) rats were trained to lever press for water on a fixed ratio 5 (FR5) schedule of reinforcement. On days 1-8, water was available by pressing either of two levers. On days 9-16, free water was also available. On days 17-21, each time a reward was earned the associated lever was made inactive until the reward ratio was reached on the alternate lever. In the EPM study, 12 rats were tested daily for 5 minutes. Entries into and relative time spent on open arms were recorded. On days 4-13, rats received daily injections of quinpirole (0.5 mg/kg) or saline. On days 14-21, all rats received saline. Drug treatments were resumed for days 22-25.
RESULTS
Quinpirole increased contrafreeloading relative to vehicle. On the first two days requiring lever alternation, drug-treated rats earned significantly more rewards than controls. In the EPM, quinpirole reduced open arm time and entries relative to vehicle. On the 8 saline-only days, rats previously treated with quinpirole spent less time on the open arms and showed a trend toward fewer open arm entries compared to drug-naïve rats.
DISCUSSION
The greater number of rewards earned by the quinpirole-treated rats on the first two days of lever alternation indicates that the drug did not prevent learning of this new task, but rather facilitated it. The reduction in exploratory behavior shown in the EPM suggests that the drug's effects on contrafreeloading are due to an increase in anxiety rather than a heightened exploratory drive. These results support quinpirole-induced contrafreeloading as an animal model for compulsive

checking, which in humans tends to be anxiety-driven and somewhat flexible to environmental changes.

Disclosures: M.J. Frederick: None. **S.E. Cocuzzo:** None. **M.C. Hill:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.19/HH17

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: PNPD/CAPES

CNPq

Title: Amygdala modulates the behavioral and hormonal consequences of the exposure to fearful reminders in a mice model of PTSD

Authors: *R. R. SOUZA[1,2], L. M. SILVEIRA[1], A. CANTO-DESOUZA[1,2,3];
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Abstract: We have recently demonstrated that the exposure of trauma reminders in mice results in long-term altered behavioral, autonomic and hormonal responses, which resemble the fear generalization symptoms seen in posttraumatic stress disorder (PTSD). Here we investigated if the behavioral and hormonal changes induced by trauma reminders exposure are modulated by GABA-benzodiazepine receptors within the amygdala. The experimental setup consisted of exposing male Swiss mice to an intense inescapable footshock (0.5 mA/10 s) followed by three weekly exposures to a cue-reminder (CR) or to a neutral chamber (control-group).The next day, all animals were stereotaxically implanted with guide cannulas bilaterally aimed to the amygdala (AM). Six days later, mice were tested in the elevated-plus maze (EPM). Our results demonstrated that mice exposed to CR spent less time in the open arms of the EPM, when compared to control group (CR= 7.3 ± 3.3%; Control= 31.6 ± 5.9%, $p < 0.05$). Both groups presented high levels of corticosterone (nmol/L) in comparison to a naïve group (CR= 2,46 ± 0,1; Control= 2,88 ± 0,1; Naïve= 0,45 ± 0,1). Interestingly, intra-amygdala infusion of the GABA/benzodiazepine agonist midazolam (MDZ, 30 nmol/0.1µl/side), reverted the anxiety-like behavior observed in the CR-group (time in open arms; MDZ= 58.0 ± 5.6%, Vehicle= 7.3 ± 3.3%, $p < 0.05$), as well as reduced corticosterone release (MDZ= 1,45 ± 0,1; Vehicle= 2,54 ±

0,1), suggesting that the amygdala modulates the high anxiety-like responses elicited by repeated exposure to fearful reminders. Altogether, these results suggest that GABA/benzodiazepine receptors within the amygdala may be critical for fear generalization in PTSD patients.

Disclosures: R.R. Souza: None. **L.M. Silveira:** None. **A. Canto-deSouza:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.20/HH18

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant 1 R01 MH60670-11

Title: The role of sleep deprivation in the acquisition of PTSD in the rat

Authors: *W. M. VANDERHEYDEN, G. POE;
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Abstract: In today's modern world, sleep deprivation has become increasingly common. It has been suggested that this perpetual state of sleep debt may enhance our susceptibility to diseases and neurological conditions. Indeed, disturbed sleep has been hypothesized to be both a precipitating and sensitizing factor in the development of Post-traumatic Stress Disorder (PTSD). Prospective studies in humans have shown that individuals who report nightmares and fragmented sleep prior to witnessing a traumatic event have increased rates of PTSD. These studies suggest that sleep and sleep timing may be playing a critical and poorly understood role in the acquisition of the neurological condition PTSD.
In order to test the role of sleep deprivation in acquisition of PTSD we used a validated and robust rodent model of PTSD called "single prolonged stress" (SPS). SPS consisted of 2 hours of restraint, followed by 20 minutes of forced swim. After a 15 minute recovery period from the swim, animals were exposed to ether in a bell jar until loss of consciousness. The animals were then allowed 7 days in isolation. SPS has been shown to produce behavioral and hormonal responses that model PTSD. Using this rodent model of PTSD allows for analysis of behavior while allowing for the role of sleep status and PTSD.
Ten, Male, Sprague Dawley rats (300-350g) were used. Five animals were REM sleep deprived for 3 consecutive days for 6 hours during their primary sleep phase using the inverted flower pot method. The other 5 animals were allowed to sleep undisturbed in their home cage. After 3 consecutive days of sleep deprivation or normal sleep, all ten animals underwent SPS followed by a fear conditioning paradigm. This fear conditioning paradigm assessed their ability to recall a

fear associated memory. Briefly, animals were fear conditioned to anticipate a 1 mAmp footshock paired to an auditory tone. In order to extinguish this fear associated memory, 24 hours later, animals were placed in a new environment and heard 30 tones without receiving foot-shock. Another 24 hours later, the animals were placed back into the environment where the memory was extinguished to assess recall of the fear extinguished memory. Previous work has shown that SPS treated rats have difficulty extinguishing this memory and freeze more on this last day.
Three days of acute REM deprivation prior to SPS exposure significantly reduced the level of freezing that occurred on the recall day. These data support the idea that sleep timing and quality may play an important role in regulating the acquisition of PTSD.

Disclosures: W.M. Vanderheyden: None. **G. Poe:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.21/II1

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH R074697

Support from Center of Excellence for Stress and Mental Health

Title: Transient forebrain-specific CRF overexpression during early life increases vulnerability for PTSD-like symptoms in adulthood

Authors: *M. TOTH[1,2], M. GROSS[2], I. M. MANSUY[3], E. MERLO-PICH[4], R. ADAMEC[5], V. B. RISBROUGH[1,2];
[1]Psychiatry, UCSD, La Jolla, CA; [2]Ctr. of Excellence for Stress and Mental Hlth., VA, La Jolla, CA; [3]Swiss Federal Inst. of Technol., Zurich, Switzerland; [4]Addiction & Sleep Disorders DPU, Neurosciences CEDD, GlaxoSmithKline Medicines Res. Ctr., Verona, Italy; [5]Dept. of Psychology, Mem. Univ., St John's, NL, Canada

Abstract: Early life stress is a significant risk factor to develop anxiety disorders including post-traumatic stress disorder (PTSD), however, the underlying neurobiological mechanisms are still not well understood. Corticotropin releasing factor (CRF) is a major regulator of the autonomic, endocrine and behavioral response to stress. CRF concentration is elevated in human subjects diagnosed with PTSD or childhood trauma history. Hence, one can hypothesize that CRF may mediate some long-term detrimental effect of early life stress. To determine the causal

contribution of increased CRF signaling during childhood to increase susceptibility for PTSD, we induced transient CRF overexpression (CRFOE) in mutant mice during prepubertal periods and tested their response to the predator exposure model of PTSD in adulthood. Using a forebrain-restricted reverse-tetracycline system, CRFOE was restricted to forebrain regions to exclude changes of corticosterone concentrations. Developmental CRFOE increased arousal (increased startle reactivity and decreased startle habituation and prepulse inhibition) regardless of predator exposure. Predator stress induced enduring avoidance behavior in a sex-dependent manner. Male mice exposed to both developmental CRFOE and predator stress exhibited increased avoidance behaviors compared to groups receiving predator stress alone, suggesting males require a "double hit", i.e. both developmental CRF exposure and "trauma" exposure to induce a PTSD-like phenotype. Female mice however showed increased avoidance behavior regardless of CRFOE during development, suggesting "trauma" alone is sufficient to induce a PTSD-like phenotype in females. Our findings suggest (1) that increased CRF signaling during development is a significant vulnerability factor for PTSD; and (2) support the double hit hypothesis for PTSD vulnerability in males.

Disclosures: M. Toth: None. **M. Gross:** None. **I.M. Mansuy:** None. **R. Adamec:** None. **V.B. Risbrough:** None. **E. Merlo-Pich:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ldt, GlaxoSmithKline.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.22/II2

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R21MH083188

Georgia Regents University

Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development

Title: Preexisting differences in the expression of plasticity-related immediate-early genes in the medial prefrontal cortex in a rat model of PTSD

Authors: K. M. BUNTING[1,2], G. PEREZ[2], R. I. NALLOOR[2], *A. I. VAZDARJANOVA[1,2]; [1]VA MEDICAL CENTER, AUGUSTA, GA; [2]Dept. of Pharmacol. and Toxicology, Georgia Regents Univ., Augusta, GA

Abstract: Post-Traumatic Stress Disorder (PTSD) is an anxiety disorder which manifests as generalized anxiety and elevated startle response accompanied by flashbacks and intrusive memories for a traumatic event. Individual susceptibility to developing PTSD exists, because it is manifested in only a portion of individuals exposed to the same traumatic event. Our research focuses on elucidating preexisting differences in the function of the limbic system and assessing whether they are risk factors for developing PTSD. We evaluate functional plasticity by combining behavioral testing in a rat model of susceptibility to a PTSD-like behavioral phenotype (Nalloor, Bunting and Vazdarjanova, 2010), with assessment of expression of two plasticity-related immediate-early genes (IEGs), *Arc* and *Homer 1a (H1a).*

Previously, we reported that rats classified as susceptible (Sus) prior to a traumatic event in our model show impaired fear extinction and lasting elevation in acoustic startle responses compared to rats classified as resistant (Res). Sus rats also have altered hippocampal IEG expression patterns prior to exposure to a traumatic event. Here we report that preexisting functional differences between Sus and Res rats are not restricted to the hippocampus, but are also present in the medial prefrontal cortex. After behavioral classification, Sus and Res rats were allowed to explore and learn about a novel place twice, with a 30 min rest between the exploration events. Their brains were collected for *Arc/H1a* catFISH (cellular-compartmental analyses of temporal activity using fluorescence *in*-situ hybridization) immediately after the second event.

While there were no significant gross differences in exploration behavior between Sus and Res rats, the patterns of IEG expression differed between the two groups. Res rats showed a consistent IEG expression in infralimbic cortex (IL or area 25) during both behavioral events. In contrast, Sus rats had a smaller IEG-expressing neuronal ensembles during both events, with a larger degree of the effect seen during the second event. No between group differences were observed in the prelimbic cortex (PreL or area 32): the second event induced a larger ensemble size than the first event in both groups.

These differences in patterns of IEG expression suggest that Sus rats have deficits in encoding and integration of sensory information in the limbic cortex (area 25) which exists before they experience a PTSD-inducing event. As area 25/IL is known to be necessary for fear memory extinction, an impairment found in PTSD patients, our findings further suggest that impaired functional plasticity in this region is a risk factor for PTSD.

Disclosures: K.M. Bunting: None. **G. Perez:** None. **R.I. Nalloor:** None. **A.I. Vazdarjanova:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.23/II3

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NSC Poland Preludium Grant 2011/03/N/NZ4/03504

Title: Sex differences in an animal model of PTSD

Authors: *M. MIKOSZ[1], K. ROKOSZ[2], W. SZADZINSKA[2], K. KONDRAKIEWICZ[2], E. KNAPSKA[2];
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Abstract: Posttraumatic stress disorder (PTSD) is a relatively common disorder developed following exposure to a traumatic event, afflicting 7-12% of the population within a lifetime. Women are consistently shown to be twice as likely as men to develop PTSD. Moreover, women were shown to have different susceptibility to PTSD following a trauma, depending on their hormonal status related to menstrual cycle. Clarification of the biological mechanism underlying sex differences in the susceptibility to PTSD is necessary to design sex-specific therapies. We addressed the issue of sex differences in susceptibility to PTSD using a valid animal model of acquisition, extinction and renewal of conditioned fear. We addressed two questions: (1) what is the impact of estrus cycle phase on fear memory acquisition, extinction and recall and (2) whether there are sex- and estrus cycle-dependent differences in the activation of the fear-controlling circuit, measured using c-Fos expression as a marker of neuronal activity. We hypothesized that hormonal status would influence memory formation and recall at all stages of the behavioral procedure. Therefore, we monitored estrus cycle phases in female rats and performed the study in a matrix design, carrying out fear conditioning, extinction and fear/extinction memory recall in either estrus or metaestrus phase. Males and gonadectomized animals of both sexes have been trained at corresponding time intervals. While estrus cycle phase of female rats during fear conditioning did not affect their behavior in response to initial CS's of the extinction training, hormonal status during both extinction and fear/extinction memory recall affected animals' freezing rates. Both intact and castrated males, as well as castrated females, showed higher freezing rates than intact females when exposed to CS's presented in the conditioning context. In groups of females differing significantly in their freezing rates during fear/extinction memory recall, the activity of the fear-controlling circuit was studied. Here we present the data concerning c-Fos expression in the circuit comprising of the amygdala, ventral hippocampus and medial prefrontal cortex in these animals.

Disclosures: M. Mikosz: None. **K. Rokosz:** None. **W. Szadzinska:** None. **K. Kondrakiewicz:** None. **E. Knapska:** None.

Poster

543. Behavioral Effects of Stress

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.24/II4

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: Department of Defense Grant W81XWH-10-1-0925

Title: Effects of heavy alcohol use on frontal cortex activity and behavior during emotional processing in veterans with PTSD

Authors: *G. L. FORSTER[1], D. OLSON[1], L. A. BAUGH[1], J. M. HANSEN[1], R. GAHER[1], J. SIMONS[1], V. MAGNOTTA[2];
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Abstract: Emotionally-significant stimuli activate frontal cortical regions such as the anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC), thought to inhibit the activity of subcortical structures involved in the expression of fear and anxiety states. Many imaging studies suggest that posttraumatic stress disorder (PTSD) is associated with hypofunction of these frontal cortical areas. For example, Vietnam veterans with PTSD fail to show rostral ACC activation during the emotional counting stroop task (ECST) in conditions when combat-related words are presented (e.g. Shin et al., 2001). However, other studies show increased activation of the mPFC or ACC with combat-related imagery in veterans with PTSD (e.g. Hopper et al., 2007; Morey et al., 2008). Differences between studies have been attributed to variation in the tasks used, the chronicity of PTSD (i.e. Vietnam verses post 9/11 veterans), differences in PTSD symptomology, and regional differences in emotional processing within the ACC and mPFC. Another factor that could influence frontal cortex function is alcohol use. As many as 50-85% of individuals with PTSD have a comorbid alcohol use disorder, and heavy alcohol use is associated with reduced ACC activation during evaluation of emotional facial expressions. Therefore the current study aimed to determine behavioral and neural reactivity in response to the ECST in post 9/11 veterans with and without heavy alcohol use. Forty-one male and female right-handed veterans of Operations Enduring Freedom and Iraqi Freedom (OEF/OIF) were recruited, screened for neurological and health issues that would preclude them from the study, and were assessed for combat intensity, PTSD, alcohol use and alcohol dependence. Veterans participated in functional magnetic resonance imaging (fMRI) scanning while performing the ECST adapted for OEF/OIF veterans. At the conclusion of the scanning, participants provided arousal ratings for words comprising the ECST. Veterans with PTSD exhibited increased reaction times to combat-related words in the ECST, and provided higher arousal and negative ratings for combat words compared to veterans without PTSD. Analysis of imaging data thus far suggests increased activation of the ACC in response to combat-related words of veterans with PTSD sans heavy alcohol use or dependence, as compared to control veterans. Overall, the findings will assist in clarifying the relationship between PTSD and ACC function during

emotional processing in veterans, and will provide insight to the impact of alcohol on this relationship.

Disclosures: G.L. Forster: None. **D. Olson:** None. **L.A. Baugh:** None. **J.M. Hansen:** None. **R. Gaher:** None. **J. Simons:** None. **V. Magnotta:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.25/II5

Topic: F.02. Animal Cognition and Behavior

Support: VISN18 New Investigator Award Department of Veterans Affairs

NSF1117303

Title: Pharmacological and deep brain stimulation treatments in rodent models of post-traumatic stress disorder

Authors: E. JANEZIC[1], R. LAHOOD[1], D. STIDD[2], J.-P. LANGEVIN[4], *E. D. FRENCH[3], J.-M. FELLOUS[1];
[1]Psychology, [2]Neurosurg., [3]Univ. of Arizona, Tucson, AZ; [4]Neurosurg., Southern Arizona VA Hlth. Care Syst., Tucson, AZ

Abstract: Post-traumatic stress disorder (PTSD) is a psychiatric disorder marked by symptoms of re-occurrence, avoidance and hyperarousal that afflicts 8-10% of adults (Kessler et al., 1995). Currently only two FDA approved drugs, sertraline and paroxetine, are available for its treatment. However, patients treated with these selective serotonin reuptake inhibitors (SSRIs) rarely exceed response rates of 60% while less than 20-30% of them attain complete remission (Berger et al., 2009). Hyperactivity in the amygdala and a decrease in system-wide dopamine levels have been identified as neurophysiological changes in animal models of PTSD. Our study explores alternative treatments for PTSD by targeting dopamine pathways through pharmacological intervention (nicotine and oxytocin) as well as amygdala involvement via deep brain stimulation. Using a footshock model of PTSD in rats (Corral-Frias, et al, 2012), oxytocin was peripherally administered (SC 0.25mg/kg) immediately after footshock and after three subsequent situational reminders. Nicotine was administered through osmotic mini-pumps (6 mg/day for 17 days) starting 8 hours before the footshock. Deep brain stimulation (DBS) of the amygdala (4 hours/day for 14 days) was tested using a ball-burying PTSD model (Mikics et al., 2008).

The oxytocin group showed a significant increase from baseline in time spent in the center of the open field, and a significant difference from the saline treated group, along with a trend to spend more time in the white side of the black and white box. Nicotine did not produce significant differences from the saline control group in any test. Two weeks following DBS of the amygdala, the trauma associated object (ball) was buried less compared to the paroxetine-treated animals. However the DBS group exhibited greater background anxiety than the paroxetine group as measured by the elevated plus maze. Results from ongoing experiments involving chronic oxytocin treatment using osmotic minipumps will also be presented. The results from our animal model indicate that oxytocin may effectively attenuate the consolidation of the memory, but DBS of the amygdala may be more effective. Thus, oxytocin should further be evaluated as a treatment for PTSD. The decrease in anxiety from smoking as reported by PTSD patients was not confirmed in our animal model at this time.

Disclosures: E. Janezic: None. **E.D. French:** None. **R. LaHood:** None. **D. Stidd:** None. **J. Fellous:** None. **J. Langevin:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.26/II6

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NSF Grant IOS-0914386

NIH Grant GM093854

Title: Sexual Conspecific Aggressive Response (SCAR): A model for sexual abuse and trauma in women during adolescence

Authors: *K. E. TOBON[1,2], G. DIFEO[1], M. CHANG[1], T. J. SHORS[1];
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Abstract: Sexual trauma during adolescence is extremely stressful and can result in post-traumatic stress disorder (PTSD) and/or major depressive disorder during adulthood (Shea et al, 2005). Women are especially vulnerable to these disorders and more likely to be sexually traumatized (Kessler et al, 2003, & Parker et al, 2010). In addition, the female brain is especially sensitive to stressful life experience (Shors et al., 2009). Little is known about the neuronal consequences of abuse because there is no established animal model. To meet this need, we

developed a model known as SCAR, which stands for Sexual Conspecific Aggressive Response. During puberty, a young female rat was exposed to an adult male for 30 minutes periods every third day for 22 days, for a total of 8 exposures. During each experience, the male pinned the female ~10 times with nearly 30 ano-genital sniffs. As the age of the juvenile female increased, the number of sniffs and mounts upon the juvenile female also increased. These behaviors indicate socially subjugation of the pubescent female, behaviors that were not highly expressed in adult rats of either sex. We hypothesize that the pubescent females will learn to avoid the context where the subjugation occurred, even as adults. To test this hypothesis, we employed a two arm chamber with specific visual and textile cues to differentiate the arms. Juvenile male or females were place in one designated arm of this novel chamber either by themselves, or with either an adult experienced breeder male or breeder female for the SCAR experience described above. At the end of the SCAR procedure (postnatal day 57), experimental animals were allowed to freely explore both arms of the two-arm chamber. The data suggest that the SCAR experience during pubescence decreased exploratory activity in females as adults. These and other data related to this novel model for early sexual trauma in females will be presented.
Funding: This study was supported by NSF (IOS-0914386) to TJS and IRACDA, NIH (GM093854) to KT.

Disclosures: K.E. Tobon: None. **G. DiFeo:** None. **M. Chang:** None. **T.J. Shors:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.27/II7

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Title: Effect of pregabalin in the extinction re-call of fear conditioned exacerbated by neonatal maternal separation with early weaning in C57BL6/N mice

Authors: *S. M. MARCELLO;
LOC. PISCINAMANNA, PULA, Inst. Di Farmacologia Traslazionale CNR UOS Pula (CA), PULA (CA), Italy

Abstract: Post traumatic stress disorder (PTSD) can occur following to strong traumatic events. Nevertheless, only a few of the people who experiencing traumatic events get PTSD. Thus, it were identified pre-traumatic risk factors that can explain the development of the disorder. Among these, adverse childhood experiences, such as periodic maternal separation, can evolve in an increase of vulnerability factors toward PTSD diagnosis. Fear conditioning has been

extensively used to model anxiety disorders, including PTSD and to develop new therapies but “normal” animals are usually employed, however, it would be of great utility to use animals with intrinsec vulnerability to the disease. Therefore, we wanted to recreate a validated experimental "platform" including a protocol for neonatal maternal separation with early weaning (MSEW), on which the effect of anxiolytic treatment on fear conditioned extinction re-call will be tested. In this study we investigated, by fear conditioning paradigm, if 30 mg/kg chronic therapy whit pregabalin, would improve fear memory during extinction re-call test respect to chronic therapy whit the anticonvulsant/anxiolytic drug diazepam (1mg/kg i.p.), respect to chronic therapy whit the anticonvulsant/anxiolytic drug diazepam (1mg/kg i.p.), in C57Bl6/N adult mice previously exposed to the MSEW, respect to the mice brought in standard conditions. When adults, C57/BL6/N mice were tested prior in fear conditioning, in order to allow the learning and the expression of cue fear conditioned. 72 hours after fear conditioning learning, the mice were exposed to extinction training to allow and assess the expression of fear-conditioned extinction. The same day, the mice were subdivided in three groups and for 21 days with 30-mg/kg pregabalin or two crescent diazepam doses (0,25-1mg/kg) or vehicle chronically were treated. So, the day of last drug administration, the fear extinction re-call test was assessed. We found that the MSEW model, coupled with the fear-conditioning paradigm could provide a degree of face validity for the model of PTSD, by reproducing the aspect of vulnerability of desease. Finally, the chronic pregabalin treatment improved the extinction re-call respect to vehicle-treated mice and diazepam-treated mice, previously stress-sensitisation by MSEW. These results suggest a new pharmacological therapy adjuvant to cognitive behaviour therapy (CBT) for PTSD.

Disclosures: S.M. Marcello: None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.28/II8

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R21MH091445

Klarman Family Foundation Grant Program in Eating Disorders Research

NYU Dean's Undergraduate Research Fund

NIH P30 EY13079

Title: Noradrenergic fibers in the cerebellar cortex of adolescent female rats become more varicose following 7 days of voluntary wheel running

Authors: *K. TATEYAMA[1,2], H. NEDELESCU[3,4], T. CHOWDHURY[2], G. WABLE[2], G. ARBUTHNOTT[3], C. AOKI[2];
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Abstract: The Norepinephrine (NE) system plays an important role in acquisition of motor learning via ascending projections to the cerebellum, mostly originating from the locus coeruleus, an area involved in arousal, vigilance, and responses to stress and panic. Despite the fact that NE fibers innervate practically all parts of the cerebellar cortex, including the molecular layer where the dendritic tree of Purkinje cells reside, it has received little attention. The goal of our study was to determine whether NE innervation is altered in response to mild voluntary exercise on the wheel (ca 5 km/day), and whether this is different from the voluntary hyper-exercise (ca 15 km/day) that is evoked by the stressful situation of food restriction in a paradigm called activity-based anorexia (ABA). Previous studies have shown that hyper-exercise exhibited by ABA rats is maladaptive, in that it causes negative energy balance to the point of death, unless removed from the ABA-inducing environment. To quantify the effect of exercise on NE innervation, three rats were singly housed in the presence of a running wheel starting P36 until P44 (EX group), while four other rats were ABA-induced by allowing voluntary wheel access and limiting food availability to 1 hr/day. In addition, three other rats were singly housed without a wheel but with ad libitum food access (CON group) for the matching ages to obtain baseline values. We used the primary antibody dopamine-β-hydroxylase (DBH from EugeneTech) and subsequently visualized NE fibers and their varicosities with 3,3'-diaminobenzidine (DAB) as the immunolabel. We acquired image stacks of the DAB labeled NE fibers using a confocal microscope equipped with a Photo Multiplier Tube (PMT) for digitization of the microscopic image. Next, using the Neurolucida Software (MBF Bioscience), we traced each NE fiber and added a marker at each encountered varicosity. The inter-varicosity distances were 9.01 ± 0.8 um for the CON group (N =3) and reduced to 6.56 ± 0.2 um for the EX group (N = 3) and also reduced to 6.93 ± 0.3 for the ABA group (N =4). In addition, the total number of varicosities were increased for both the EX and ABA group. This difference across the groups suggests that voluntary wheel running during adolescence increases the amount of NE released in the molecular layer of the cerebellum. Interestingly, the ABA group demonstrated shorter NE axonal total lengths and smaller number of axons, while the EX group exhibited longer fibers and a larger number of NE axons. This latter finding suggests that NE fiber growth may be stunted by hyper-EX and/or food restriction but is enhanced by mild EX.

Disclosures: K. Tateyama: None. **H. Nedelescu:** None. **G. Arbuthnott:** None. **C. Aoki:** None. **T. Chowdhury:** None. **G. Wable:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.29/II9

Topic: F.01. Human Cognition and Behavior

Support: The Trust for the Meditation Process

Title: Use of a modified Trier Social Stress Test to assess an undergraduate meditation course

Authors: A. C. HEUERMAN[1], G. N. CHAVEZ[1], M. C. GREEN[1], M. HUERTA[1], J. OV[1], P. L. OVERTON-HARRIS[1], F. GRACE[2], C. M. KO[3], *L. E. OLSON[1];
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Abstract: Anecdotal evidence suggests that undergraduate students who take the University of Redlands course Seminar on Compassion exhibit behaviors consistent with improved academic success: enhanced focus, goal-setting, resilience to stress, and general well-being. The course includes the practice of contemplative methods designed to generate compassion, such as those taught by Mother Teresa, Gandhi, and the Dalai Lama. We are using psychological measures of a variety of clinical and nonclinical outcomes such as anxiety, depression, self-compassion, compassion for others, locus of control, and general well-being. Additionally, we are measuring the physiological stress response in the laboratory. We modified the Trier Social Stress Test (TSST) in an attempt to closely mimic academic stress. We replaced the interview portion of the TSST with verbal GRE analogy questions, and included a neutral question period to control for the effect of speaking. Our pre-meditation course data shows that our altered version of the TSST was effective in reducing parasympathetic nervous activation (reduced high frequency heart rate variability), and increasing systolic and diastolic blood pressure, heart rate, and galvanic skin response. It did not induce a surge in salivary cortisol. This provides baseline physiological data which can be correlated to academic success before and after the meditation-based Seminar on Compassion course, so the effects of this course can be quantitated over time.

Disclosures: A.C. Heuerman: None. **M.C. Green:** None. **L.E. Olson:** None. **M. Huerta:** None. **G.N. Chavez:** None. **P.L. Overton-Harris:** None. **C.M. Ko:** None. **J. Ov:** None. **F. Grace:** None.

Poster

543. **Behavioral Effects of Stress**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 543.30/II10

Topic: C.17. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH MH085069-02

NSF GRF

Sigma-Xi

Title: Sex differences in mu-opioid receptor regulation of reversal learning in the California mouse (Peromyscus californicus)

Authors: *S. A. LAREDO[1], M. Q. STEINMAN[2], C. F. ROBLES[3], E. FERRER[3], G. D. GREENBERG[4], A. LAMAN-MAHARG[4], B. C. TRAINOR[5];
[1]Psychology, Animal Behavior, [2]Psychology, MCIP, [3]Psychology, [4]Psychology, Neurosci., [5]Psychology, Animal Behavior, MCIP, Neurosci., Univ. of California Davis, Davis, CA

Abstract: Cognitive flexibility, the ability of an animal to change its behavioral patterns to better adapt to the surrounding environment, is widely known to be impaired following stressful situations in male rodents. The literature surrounding cognitive flexibility following stress in females, however, remains largely unexplored. Psychological stressors often rely on intra-specific aggression, and in most rodent species, females do not display intra-specific aggression. Utilizing a monogamous rodent, the California mouse, we are able to employ a potent psychological stressor, social defeat, in both males and females since both sexes demonstrate territorial aggression. We previously showed that following social defeat, male mice demonstrated impaired reversal learning in a Barnes Maze paradigm, but females did not. Further analyses indicated that the reason for poor male performance was due to perseverative behaviors, as stressed males were more likely to return to the original target location during reversal trials. Previous studies have demonstrated that the mu-opioid receptor (MOR) is modulated by social defeat stress, and can play a role in spatial learning behavior. When we injected control mice with the MOR antagonist beta-funaltrexamine (β-FNA), they showed a similar phenotype in reversal learning as did mice that had undergone social defeat. Males injected with β-FNA also showed a perseverative phenotype, and returned to the acquisition hole more often than females injected with β-FNA. Based on these data we tested whether defeat decreased MOR gene expression in the hippocampus and prefrontal cortex. Relative gene expression of MOR was not different between sexes or treatments in any brain region examined. Unexpectedly, pro-enkephalin mRNA (a precursor to an MOR ligand), was significantly upregulated in the dentate gyrus of stressed females. The current data suggest that mu-opioid systems are affected by defeat, but not likely at the level of gene expression in the hippocampus or prefrontal cortex. Ongoing studies are therefore examining the effects of defeat on the number

of available MOR binding sites in the hippocampus and prefrontal cortex via autoradiography. This work has important clinical applications for patients suffering from PTSD and depression since a persistent symptom of these diseases is perseveration on negative thought processes. These data thus allow a better understanding of how mental disorders manifest differently in males and females.

Disclosures: S.A. Laredo: None. **M.Q. Steinman:** None. **C.F. Robles:** None. **E. Ferrer:** None. **G.D. Greenberg:** None. **A. Laman-Maharg:** None. **B.C. Trainor:** None.**Poster**

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.01/II11

Topic: C.18. Drugs of Abuse and Addiction

Support: NIAAA/NIH AA017243 (to LA)

P50AA11199 (to HT)

USC School of Pharmacy

Title: P2X7 receptor-driven neuroinflammation plays a role in causing alcohol related brain damage

Authors: *L. ASATRYAN[1], S. KHOJA[1], K. RODGERS[1], H. TSUKAMOTO[2], R. L. ALKANA[1], D. L. DAVIES[1];
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Abstract: The mechanisms leading to brain damage caused by chronic alcohol abuse (alcohol related brain damage or ARBD) remain poorly understood. Recent genetic studies using post-mortem human brain tissue samples and animal models of drinking suggest that neuroinflammation is an important path leading to ARBD. ATP-gated purinergic P2X7 receptors (P2X7Rs) are expressed in neuroimmune cells such as glia and astrocytes and play a critical role in microglia activation and release of pro-inflammatory cytokine IL-1β, a known mediator of neurodegeneration. As such, growing evidence implicates P2X7Rs in the pathophysiology of neurodegenerative and neuropsychiatric disorders. We began testing the hypothesis that P2X7Rs contribute to alcohol-induced neuroinflammation leading to ARBD. Our initial studies found that alcohol can differentially alter P2X7R expression in alcohol-sensitive brain regions depending on the length and amount of alcohol exposure. However, the low blood ethanol concentrations (BECs) used in these studies did not produce neuroinflammatory responses. To overcome this limitation, the current study used mouse models of alcohol exposure that are characterized by

BECs that reach as high as ~ 400 mg/dL (or ~ 100 mM) and that are known to produce significant liver damage resembling human alcoholic liver disease (provided by Southern California Research Center for ALPD and Cirrhosis). We predicted that these models will also lead to increased neuroinflammation. The base model (Hybrid) is generated by intragastric ethanol diet infusion combined with ad libitum feeding of a Western high fat diet for a period of 8 weeks. The effect of ethanol is further amplified by addition of weekly binge events in the Hybrid+Binge model. We tested the expression of P2X7Rs in alcohol-sensitive brain regions from Hybrid and Hybrid+Binge models and compared to those from pair-fed Control animals (given isocaloric dextrose and diet). We found a significant increase in P2X7R expression in Hippocampus, Striatum and Midbrain for Hybrid+Binge as compared to the Control. There was also a significant increase in P2X7R levels in Hybrid compared to the Control in Striatum. Histological changes that occurred in the same brain regions were typical for neuroinflammation in Hybrid and Hybrid+Binge models. The results support the hypothesis and suggest that alcohol induced changes in P2X7R-driven neuroinflammatory processes may cause or modulate ABRD. The findings also identify P2X7Rs as potential new therapeutic targets for developing new drugs to prevent or reduce brain damage associated with chronic alcohol abuse and dependence.

Disclosures: L. Asatryan: None. **S. Khoja:** None. **K. Rodgers:** None. **H. Tsukamoto:** None. **R.L. Alkana:** None. **D.L. Davies:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.02/II12

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH, NIAAA Grant AA017234

Department of Defense

Title: Epigenetic modifications in frontal cortex of HS/Npt mice following chronic intermittent exposure to ethanol

Authors: *G. GORINI[1], S. BLOCH[1], J. C. CRABBE[2], R. A. HARRIS[1], I. PONOMAREV[1]; [1]Waggoner Ctr. Alcohol Addiction Res., The Univ. of Texas at Austin, Austin, TX; [2]Portland Alcohol Res. Center, VA Med. Center, and Dept of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Alcohol dependence results from alcohol's effects on various brain targets. Altered patterns of gene/protein expression following excessive alcohol exposure suggest molecular changes at the epigenetic level, which underlies a wide range of adaptations in brain function. Previous evidence from our laboratory showed that neuropathology and neuroadaptations contributing to alcohol addiction and dependence are, at least in part, mediated by alcohol-induced epigenetically mediated changes in gene expression. Here, we investigated the changes in global histone 3 lysine 4 tri-methylation (H3K4me3) and H3 acetylation (H3Ac) in cerebral frontal cortices (FC) of genetically heterogeneous (HS/Npt) female mice subjected to a chronic intermittent ethanol (CIE) paradigm that represents a model for alcohol dependence. Mice were given 4 weeks of 2-hour daily limited access to 15% ethanol and water, under two-bottle choice (2BC) conditions, to measure baseline ethanol drinking and preference. Mice were then exposed to 3 consecutive episodes consisting of 4 days of either ethanol vapor (CIE) or Air (Control) for 16h/day followed by 5 days of 2BC drinking. The CIE paradigm resulted in ~80% statistically significant increase in ethanol drinking during the last 5 days (CIE group compared to Control). Brains were isolated 24 hours after the last drinking session, flash-frozen and sliced, and micropunches from FC were collected for each sample. Total histone extracts were prepared from tissues of 20 Control and 19 CIE mice and levels of H3K4me3 and H3Ac were measured using colorimetric assays. In our initial experiments, CIE exposure induced a statistically significant decrease in H3Ac (-18%; $p<0.05$). In addition, levels of H3K4me3 were inversely correlated with ethanol consumption across all samples ($p=0.05$). We are currently replicating these assays and profiling samples from the same animals to investigate changes in gene expression in response to the CIE treatment. These results advance our understanding of the complex relationship between genetic, epigenetic, and environmental factors affecting gene expression in alcohol dependence.

Disclosures: G. Gorini: None. **S. Bloch:** None. **R.A. Harris:** None. **I. Ponomarev:** None. **J.C. Crabbe:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.03/II13

Topic: C.18. Drugs of Abuse and Addiction

Title: Changes in the expression of protein phosphatase 2A in ethanol relapse

Authors: *K. MIZUO, R. KATADA, S. OKAZAKI, S. WATANABE;
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Abstract: Recent studies suggest that the epigenetic regulation of gene expression such as histone acetylation may play an important role in alcohol dependence. We previously found that the decrease of histone H3 acetylation and increase of histone deacetylase 5 (HDAC5) in ethanol relapse. Several studies have shown that protein phosphatase 2A (PP2A) may regulate the HDAC5 nuclear shuttling. In the present study, we investigated the expression of PP2A in the mouse ethanol relapse model. Mice were treated with liquid diet containing ethanol for 10 days. Using the escalating ethanol dosage schedule, the mice were fed the ethanol diet as follows: 1st day: 1 w/v%; 2nd and 3rd day: 3 w/v%; 4th and 5th day: 4 w/v% and from the 6th to 10th day: 5 w/v% ethanol diet, respectively. The control mice were given the same volume of ethanol-free liquid diet with glucose substituted in isocaloric quantities for ethanol. The mice chronically treated with ethanol revealed severe withdrawal signs. Ten days after withdrawal, we performed a conditioned place preference to evaluate ethanol relapse. At the dose of 0.5 g/kg of ethanol, which produced neither preference nor aversion in control group, the alcohol group showed significant rewarding effects. Under these conditions, the mice were killed by decapitation and the limbic forebrain (containing nucleus accumbens) was dissected. PP2A was increased in limbic forebrain at ethanol relapse state. Our findings suggest that the increase in PP2A caused dephospholylation of HDAC5, resulting in the increase of HDAC5 nuclear transport in ethanol relapse.

Disclosures: K. Mizuo: None. **R. Katada:** None. **S. Okazaki:** None. **S. Watanabe:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.04/II14

Topic: C.18. Drugs of Abuse and Addiction

Support: University of Florence

Compagnia di San Paolo

Tuscany Region

Title: Neurotoxicity and synaptic transmission alterations in immature and mature rat organotypic hippocampal slice cultures exposed to ethanol

Authors: *D. PELLEGRINI-GIAMPIETRO[1], E. GERACE[1], E. LANDUCCI[1], T. SCARTABELLI[1], F. MORONI[2], G. MANNAIONI[2];

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Abstract: Chronic ethanol consumption causes persistent molecular alterations and morpholgical injury in brain cells and affects the maturation of neuronal circuits by mechanisms that are not fully understood. To comprehend the mechanisms by which ethanol modulates excitatory glutamatergic synaptic transmission, we studied the expression of pre (vGlut1, vGlut2, CB1 receptor, synaptophysin) and postsynaptic (GluA1, GluA2, NR2A, NR2B) proteins, the electrophysiological changes and the ultrastructural modifications in immature (2 days in vitro) or mature (10 days in vitro) organotypic rat hippocampal slices exposed to chronic ethanol (150 mM ethanol for 7 days) or following ethanol withdrawal (150 mM ethanol for 7 days following 24 h ethanol withdrawal). We observed no changes in the expression levels of vGlut1, vGlut2 and CB1 receptor proteins neither in immature nor in mature hippocampal slices. Immature organotypic hippocampal slices showed a decreased in synaptophysin, GluA1 and NR2A expression levels both in chronic ethanol and ethanol withdrawal thus suggesting an impairment in glutamatergic synaptic transmission. On the other hand, mature organotypic hippocampal slices showed a significant increase in GluA1 expression following ethanol withdrawal which may underlie increased toxicity to glutamate. Whole cell patch-clamp recordings both in chronic ethanol and ethanol withdrawal showed a significant reduction in the frequency but not in the amplitude of spontaneous excitatory post synaptic currents (sEPSCs) and a significant increase in the amplitude but not in the frequency of sEPSCs in CA1 pyramidal cells from immature and mature organotypic hippocampal slices, respectively. Finally, electron microscope analysis revealed that immature organotypic hippocampal slices exposed chronically to ethanol showed a clear disorganization of dendritic microtubuli, while mature hippocampal slices displayed an apoptotic cell death pattern after chronic and ethanol withdrawal. These findings suggest that chronic ethanol treatment promotes abnormal glutamatergic synaptic transmission especially in immature hippocampal neurons and that ethanol withdrawal leads to cell death of mature but not immature CA1 pyramidal cells.

Disclosures: D. Pellegrini-Giampietro: None. **E. Gerace:** None. **E. Landucci:** None. **T. Scartabelli:** None. **F. Moroni:** None. **G. Mannaioni:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.05/II15

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant AA016789

NIH Grant AA10760

NIH Grant AA13519

NIH Grant AA020245

Department of Veterans Affairs and the Department of Defense Grant

NIH AA07468

OHSU Graduate Research Scholar Fellowship

Title: Mice selectively bred for High Drinking in the Dark exhibit reduced sensitivity to the ataxic and hypnotic effects of ethanol but do not differ in acute functional tolerance relative to progenitor HS/Npt mice

Authors: *K. A. CORDERO[1,2], B. M. FRITZ[2], A. M. BARKLEY-LEVENSON[1], P. METTEN[1], J. C. CRABBE[1], S. L. BOEHM, II[2];
[1]Behavioral Neurosci., Portland Alcohol Res. Center, Oregon Hlth. & Sci. University, and the VA, Portland, OR; [2]Psychology, Indiana Alcohol Res. Center, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Initial sensitivity to ethanol and the capacity to develop acute functional tolerance (AFT) to both ataxia and hypnosis may influence the amount of alcohol an individual consumes and may also mediate the development of alcoholism. The goal of the current study was to assess sensitivity and AFT to the ataxic and hypnotic effects of ethanol in the first replicate of High Drinking in the Dark (HDID-1) mice. This mouse line is selectively bred for high blood ethanol concentrations following limited access to ethanol in the Drinking in the Dark (DID) paradigm. Mice from the HS/Npt progenitor stock were tested as controls. Ataxia was assessed by the static dowel task which requires animals to balance on a wooden dowel. Ethanol-induced hypnosis was assessed by the method of Ponomarev and Crabbe (2002), using modified restraint tubes to measure the loss of righting reflex (LORR). HDID-1 mice exhibited reduced initial sensitivity to both ethanol-induced ataxia ($p < 0.001$) and hypnosis ($p < 0.05$) as evidenced by significantly higher BEC values at loss of function in both tasks relative to HS/Npt mice. AFT was calculated for both tasks by subtracting the BEC at loss of function from the BEC at recovery. The dowel test yielded no line differences in AFT, but HS/Npt mice developed slightly greater AFT to ethanol-induced LORR than HDID-1 ($p < 0.05$).These results suggest that HDID-1 mice exhibit blunted initial sensitivity to ethanol relative to Hs/Npt mice which may influence their subsequent high ethanol intake in the DID paradigm.

Disclosures: K.A. Cordero: None. **B.M. Fritz:** None. **A.M. Barkley-Levenson:** None. **P. Metten:** None. **J.C. Crabbe:** None. **S.L. Boehm:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.06/II16

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH/NIAAA INIA West Consortium Grant U01-AA013498

NIH/NIAAA Grant AA020893

NIH Grant GM 101279

Title: Evaluation of TLR4 inhibitor T5342126 as a potential candidate drug for treatment of alcoholism

Authors: *M. BAJO[1], A. J. ROBERTS[2], H. YIN[3], L. N. CATES[2], T. NADAV[2], K. CHENG[3], S. COULUP[3], S. MADAMBA[1], G. R. SIGGINS[2], M. ROBERTO[1];
[1]CNAD, [2]Mol. and Cell. Neurosci., Scripps Res. Inst., La Jolla, CA; [3]BioFrontiers Inst., Univ. of Colorado Boulder, Boulder, CO

Abstract: The toll-like receptor 4 (TLR4) inflammatory pathway has been shown to play a critical role in enhanced alcohol consumption and the progression to alcoholism. Thus, inhibitors of TLR4 have emerged as promising candidate drugs for the treatment of alcoholism. We evaluated a drug-like small-molecule compound, T5342126, that inhibits TLR4 signaling via the disruption of TLR4/MD-2 (myeloid differentiation protein-2) complex. We used C57BL/6J mice and a chronic intermittent ethanol, two bottle choice (CIE-2BC) method to induce ethanol dependence and assess T5342126 effects on ethanol drinking. We administered T5342126 (82 mg/kg, i.p.) 30 minutes prior to 2BC tests for three consecutive days during the 2BC period, and we performed testing after the 3rd and 5th ethanol exposure episodes (each episode lasting 5 days). T5342126 had no effect on ethanol drinking in control mice in any of the weeks examined. In ethanol dependent mice, T5342126 significantly decreased ethanol intake on the second day of the administration after the 3rd ethanol exposure episode (vehicle: 2.51 ± 0.29 g/kg, n = 10; and T5342126: 1.36 ± 0.42 g/kg, n = 8) as well as after the 5th exposure episode (vehicle: 2.6 ± 0.16 g/kg, n = 10; and T5342126: 1.32 ± 0.34 g/kg, n = 8). We examined withdrawal symptoms after the 4th ethanol exposure episode, by focusing on handling-induced convulsions (HICs) and body temperature. We administered T5342126 1 hour after removal from the vapor chambers on the last 3 days of the ethanol exposure, and tested for withdrawal symptoms on the final day within 12 hours post-withdrawal. In control mice, T5342126 had no significant effects on HICs or body temperature, but reduced HICs in the ethanol dependent mice

compared to ethanol-vehicle mice, reaching significance at the 6 and 12 hr time points (1.13 ± 0.13, n = 10; compared to 1.6 ± 0.16, n = 8, at both time points). We detected hypothermia in ethanol dependent mice (36.82 ± 0.20 °C, n = 10) compared to control mice (37.63 ± 0.15 °C, n = 12), and further reduction in body temperature of T5342126-treated ethanol dependent mice (35.98 ± 0.23 °C, n = 8) at 2 hours into withdrawal. These results suggest the potential efficacy of T5342126 and our treatment paradigm in reducing drinking associated with dependence as well as alcohol withdrawal severity.

Disclosures: M. Bajo: None. **A.J. Roberts:** None. **H. Yin:** None. **L.N. Cates:** None. **T. Nadav:** None. **K. Cheng:** None. **S. Coulup:** None. **S. Madamba:** None. **G.R. Siggins:** None. **M. Roberto:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.07/II17

Topic: C.18. Drugs of Abuse and Addiction

Support: 1F31AA021322-01

R01AA016852

T3207565

P01AA017056-03

P01 (AA 21099)

Tab Williams Family Fund

Title: Ethosuximide, a T-type calcium channel antagonist, as a potential treatment for alcohol dependence and withdrawal

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Abstract: Approximately 18 million Americans abuse or are dependent on alcohol which results in an economic burden of $184 billion. Individuals abusing alcohol frequently cycle between drinking and withdrawal states which can result in increased anxiety, delirium tremens, insomnia, seizures, and other adverse symptoms. Withdrawal symptoms are a major component

of relapse and represent a significant barrier to recovery. Increasingly, studies indicate a role for anticonvulsants as treatment for alcohol withdrawal symptoms as well as alcohol dependence. While the use of anticonvulsants appears to be a promising strategy, different anticonvulsants possess different mechanisms of action. Our lab has identified a disruption in the thalamic T-type calcium channel isoform, $Ca_V3.2$, during withdrawal that may underlie the generation and propagation of withdrawal symptoms. These results led us to our current hypothesis, that targeting T-channels may ameliorate alcohol withdrawal symptoms. To test this, we performed a series of experiments evaluating the use of ethosuximide, an anticonvulsant that targets T-type calcium channels. We tested the effects of ethosuximide on alcohol withdrawal seizures, anxiety measures, and alcohol consumption. Our results suggest that ethosuximide decreases alcohol withdrawal seizures, modestly reduces ethanol intake, and has little effect on general anxiety-like behavior. Further investigation is necessary to determine the role of T-channels and effectiveness of ethosuximide in alcohol withdrawal-induced anxiety. Overall these studies provide implications for targeting T-type calcium channels and the potential use of ethosuximide as a treatment option for alcohol withdrawal symptoms.

Disclosures: M. Riegle: None. **E. Carter:** None. **J. Weiner:** None. **D. Godwin:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.08/II18

Topic: C.18. Drugs of Abuse and Addiction

Support: NIAAA P50 AA010761

VA Medical Research

Title: Ethanol drinking in ethanol dependent and non-dependent mice: Role of dopamine and glutamate neurotransmission in the dorsolateral striatum

Authors: *W. C. GRIFFIN, III, H. L. HAUN, C. E. MAY, C. HAZELBAKER, V. RAMACHANDRA, C. B. HAWKINS, H. C. BECKER;
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Abstract: Repeated cycles of chronic intermittent ethanol (CIE) exposure significantly increases ethanol intake in C57BL/6J mice. Previously, we found that CIE-exposed (dependent) mice were more sensitive to the effects of reducing glutamate neurotransmission in the nucleus accumbens (NAC) on ethanol drinking than non-dependent mice. In the current study, we tested the

hypothesis that changes in glutamatergic and dopaminergic neurotransmission in the dorsolateral striatum (DLS) contribute to escalation of drinking in this model. After implanting bilateral guides above the DLS, mice were trained to drink ethanol (15% v/v) in a 2-bottle choice, limited access paradigm. After establishing stable ethanol intake, mice received 4 weekly cycles of CIE (16 hr/d for 4d) to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers, with each exposure cycle alternating with a week of limited access drinking test sessions. As expected, ethanol drinking increased in EtOH compared to CTL mice over cycles (2.8±0.2 vs 1.5±0.1 g/kg). During test periods, mice were microinjected with vehicle (PBS), the mGluR2/3 agonist LY379268 (LY; 5nmol/side) or the DRD2/3 agonist quinpirole (QU;0.5 & 1 µg/side) into the DLS 30 min prior to ethanol access. After PBS, EtOH mice continued to drink more than CTL mice (2.8±0.3 vs. 1.6±0.2 g/kg; $p<0.01$). LY reduced drinking in EtOH mice (53% decrease, $p<0.01$) and to a lesser extent in CTL mice (43% decrease; $p=0.09$) compared to vehicle. Microinjection of 0.5 µg QU into the DLS did not affect drinking in EtOH mice but slightly reduced ethanol intake in CTL mice (2.9±0.2 vs 1.4±0.2 g/kg; $p<0.01$). The higher dose of QU reduced drinking more strongly in EtOH mice compared to CTL mice (1.9±0.3 vs 1.2±0.2 g/kg, respectively). Finally, preliminary results from a microdialysis study found that extracellular dopamine levels were similar in the DLS of EtOH and CTL mice (0.86±0.2 vs 0.75±0.2 nM, respectively). Collectively, these data indicate that dopaminergic and glutamatergic neurotransmission in the DLS are altered in this model of ethanol dependence and relapse drinking. Ongoing studies are expanding the dose response range of QU & LY on ethanol drinking as well as investigating expression levels of the cognate receptors in ethanol dependent and non-dependent mice in both regions. Supported by AA010761 & VA Medical Research.

Disclosures: W.C. Griffin: None. **H.L. Haun:** None. **C.E. May:** None. **C. Hazelbaker:** None. **V. Ramachandra:** None. **C.B. Hawkins:** None. **H.C. Becker:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.09/JJ1

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH AA13983

Title: Cortical glutamate and NMDA receptors during withdrawal from intermittent alcohol

Authors: *L. S. HWA[1], A. SHIMAMOTO[1], A. J. NATHANSON[1], J. TAYEH[1], J. F. DEBOLD[1], K. A. MICZEK[1,2];
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Abstract: Alcohol withdrawal includes behavioral hyperexcitability that may be based on kindled glutamate activity. Negative affect during abstinence from a previous exposure to alcohol may be expressed by increased aggression and social impairments. This study examines glutamate and other amino acids in the medial prefrontal cortex (mPFC) during the transition from low drinking to excessive drinking. The mPFC is involved in executive control and impulsivity, and its glutamatergic efferents project to limbic structures. Outbred CFW male mice were given intermittent access to 20% w/v ethanol and water for 1-2, 4-6, or 8-10 weeks, and changes in glutamate, glutamine, and GABA were measured using in vivo microdialysis over the course of withdrawal. Levels of extracellular glutamate were significantly decreased in mice that were experiencing alcohol withdrawal from both 1-2 and 8-10 weeks intermittent access. Alcohol withdrawal induced an enhancement in glutamine levels in the mPFC and was significantly greater than glutamate levels. In an effort to challenge mPFC glutamate during withdrawal, systemic injection of the uncompetitive NMDA receptor antagonist memantine increased glutamate in mice with a history of 8 weeks of alcohol. In different groups of mice, aggression and non-aggressive interactions toward a male conspecific were also investigated. A further aim was to assess the effects of the memantine on aggression during withdrawal from ethanol. Memantine did not have a significant effect upon withdrawal aggression after 1-2 or 4-6 weeks of intermittent alcohol drinking, but a 5 mg/kg dose significantly escalated withdrawal aggression after 8-10 weeks. Mice showed differences in baseline aggression and social behavior across the transition to escalated drinking. Specifically, after 1-2 weeks of drinking mice exhibited decreased attack bites and increased contact with the intruder. After 8-10 weeks intermittent alcohol, mice showed increased baseline aggression. In accordance with the glutamate kindling hypothesis, these findings suggest that glutamine levels in the mPFC are sensitized during withdrawal, which may point to an alteration in glutamate recycling. In conclusion, we have been able to reveal several novel indicators of withdrawal from intermittent access to alcohol. Further tests will probe the exact nature of the social deficit or anxiety during withdrawal as well as measure concentrations of NMDAR in the mPFC.

Disclosures: L.S. Hwa: None. **A. Shimamoto:** None. **A.J. Nathanson:** None. **J. Tayeh:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.10/JJ2

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant R01AA020394-01

Title: KOR blockade attenuates escalated alcohol self-administration in alcohol dependent rats: Dissociation between the central amygdala and nucleus accumbens

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Abstract: Recent evidence has established that the kappa opioid receptor (KOR) / dynorphin (DYN) system is upregulated in alcohol dependence. Specifically, increased KOR signaling and DYN A-like peptide expression in the central amygdala (CeA) following alcohol dependence are thought to contribute to excessive operant alcohol self-administration and increased negative affective-like states. Previous data from this lab has established that blockade of the KOR/DYN system selectively reduces alcohol self-administration in alcohol dependent rats. Furthermore, this lab has also demonstrated that administration of a KOR antagonist into the nucleus accumbens (Acb) selectively attenuates alcohol self-administration in alcohol dependent rats. The primary purpose of this experiment was to examine a possible dissociation in alcohol consumption following KOR blockade into the CeA and Acb. Wistar rats (N = 22) were trained to self-administer alcohol and implanted with cannula guides targeting the CeA or Acb. Following recovery, subjects received four weeks of intermittent alcohol vapor exposure resulting in escalated self-administration. Testing occurred 6-8 hours into withdrawal where animals received intra-CeA or -Acb infusion of the KOR antagonist, nor-binaltorphimine (nor-BNI; 0, 2, or 6µg). A 2-way ANOVA revealed that intra-CeA and -Acb nor-BNI significantly reduced alcohol consumption in dependent animals ($p<0.05$). Another 2-way ANOVA analyzing percent change indicated that nor-BNI was more potent when infused into the Acb ($p<0.05$). This indicates a possible dissociation in regulatory mechanisms of alcohol dependence in the CeA and Acb. These results add to the field's understanding of the neurobiological mechanisms underlying alcohol dependence and highlight important areas for future research.

Disclosures: J. Kissler: None. **A. Williams:** None. **B. Walker:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.11/JJ3

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH P50 AA010761

NIH U01 AA014095

NIH U01 AA020929

NIH T32 AA007474

VA Medical Research

Title: Influence of kappa opioid receptor activation on forced swim behavior in C57BL/6J mice with a history of chronic intermittent ethanol exposure

Authors: *R. I. ANDERSON[1], L. L. SNYDER[1], R. L. MCCANN[1], J. L. HOPKINS[1], M. F. LOPEZ[1], H. C. BECKER[1,2];
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Abstract: Previously we have shown that ethanol dependence induced by repeated cycles of chronic intermittent ethanol (CIE) exposure results in significantly reduced immobility in the forced swim test (FST) compared to nondependent mice. The same results were obtained in ethanol-naïve mice after central administration of CRF. While kappa opioid receptor (KOR) activity is known to modulate behavior in the FST, little is known about how alterations in KOR activity may influence behavioral response to the FST stressor in the context of ethanol dependence. Thus, the present study was designed to examine the effects of the KOR agonist U50,488 on forced swim behavior in CIE-exposed (dependent) and control mice. Adult male C57BL/6J mice were exposed to 2 or 4 weekly cycles of ethanol vapor exposure (CIE group) or air exposure (CTL group) in inhalation chambers (16 hrs/day for 4 days). At 72 hr after final CIE (or air) exposure, subjects were administered either saline or U50,488 (10 mg/kg, i.p.) prior to a 10-min FST. A subset of subjects administered U50,488 were pretreated with the KOR antagonist nor-binaltorphimine (norBNI; 10 mg/kg, i.p.). Swim testing was repeated daily for 5 days, with saline and U50,488 injections given 10 min prior to each FST. Results indicated that behavioral responding in the FST was dependent on the number of CIE cycles, with ethanol-exposed mice demonstrating reduced immobility after 4 (but not 2) CIE cycles in comparison to CTL mice. The altered behavioral response to FST stress in 4-cycle CIE subjects was reversed in subjects administered U50,488. Further, U50,488 increased immobility in both CIE and CTL groups, an effect that was blocked by norBNI pretreatment. Given the independent and interactive roles of dynorphin (the endogenous ligand for KORs) and CRF in stress responsiveness and ethanol dependence, future work will explore interactions of CRF administration and KOR blockade on behavioral response to FST stress in ethanol dependent and control mice.

Disclosures: R.I. Anderson: None. **L.L. Snyder:** None. **R.L. McCann:** None. **J.L. Hopkins:** None. **M.F. Lopez:** None. **H.C. Becker:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.12/JJ4

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant R15 AA018213

GVSU McNair Scholars Program

Title: Kappa opioid regulation of depressive-like behavior and reward seeking during acute and protracted withdrawal from ethanol

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Abstract: Withdrawal from alcohol is often characterized by enhanced negative affect, such as symptoms of depression and anxiety, and increased reward seeking. These behavioral changes can be long-lasting in nature, which further contributes to the challenge of the long-term management of alcoholism. Recent evidence from animal models suggests that the increased activity of the dynorphin (DYN)/kappa opioid receptor (KOR) system leads to an increase in depressive-like behaviors and reward seeking following withdrawal from ethanol. The objective of the present experiments was to determine the role of the KOR system in the regulation of depression-related behaviors and saccharin reward following chronic exposure to ethanol. In the first experiment, male Wistar rats were fed an ethanol or control liquid diet for approximately four weeks. To assess the ability of the KOR antagonist nor-BNI to attenuate increases in depressive-like behavior, animals were examined in the forced swim test. Immediately after removal of the diet, rats were injected with nor-BNI (20 mg/kg, i.p.), and 24 h later, were exposed to a 10 min session of forced swim stress. The following day, rats were given a 5 min forced swim session that was recorded and examined for time spent immobile. In the second set of experiments, male Wistar rats were trained to self-administer saccharin, and following stable intake, were exposed to an ethanol or control liquid diet as described above. The ability of the nor-BNI to decrease saccharin self-administration was examined during acute withdrawal and protracted abstinence from ethanol. Following removal of the diet, rats were injected with saline, and 24 h later, were allowed to self-administer saccharin. Immediately following this initial self-administration session, animals were pretreated with nor-BNI (20 mg/kg, i.p.), and were again

allowed to self-administer saccharin 24 h later. This saccharin self-administration procedure was repeated three weeks later after rats received injections of saline and nor-BNI as described. In the forced swim test, ethanol dependent rats displayed a characteristic increase in time spent immobile compared to controls, an effect that was reversed by pretreatment with nor-BNI. nor-BNI also selectively decreased saccharin intake in ethanol dependent rats without affecting responding in controls at 48 h and 3 weeks following withdrawal. These results suggest that KOR blockade reverses depression-related behaviors and general reward seeking associated with withdrawal from ethanol following both acute and protracted periods of abstinence.

Disclosures: S.K. Jarman: None. **G.R. Valdez:** None. **A.M. Haney:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.13/JJ5

Topic: C.18. Drugs of Abuse and Addiction

Title: Drinking status, subjective response, and craving for alcohol: A translational examination of Koob's allostatic model in humans

Authors: *S. BUJARSKI, J. JENTSCH, L. A. RAY;
UCLA, Los Angeles, CA

Abstract: Translational studies in human populations stand to provide much needed clarity about the development and maintenance of alcoholism. Koob's allostatic model conceptualizes addictive pathophysiology of as a cycle of progressive neurobiological dysregulation, beginning with preoccupation and anticipation (reflecting positive reinforcement) and ending with withdrawal mediated alcohol use (reflecting negative reinforcement). To date, clinical research has not directly tested hypotheses about SR to alcohol (SR) derived from Koob's allostatic model. The present study aims to test neurobiologically-derived hypotheses about the moderating role of drinking status (i.e. heavy drinker, HDs vs. alcohol dependent, ADs) on SR in the lab (consisting of stimulation, sedation and tension relief domains) and on the relationship between SR and craving for alcohol. Thus the present study represents a translational test of key behavioral predictions derived from Koob's allostatic model. This study examined SR in HDs (n=49) vs. ADs (n=42) and tested whether HDs and ADs differ in terms of the association between SR and alcohol craving. Alcohol was administered intravenously and participants completed self-report measures of SR and craving at BrAC's of 0.02, 0.04, and 0.06 g/dl. Latent growth curve modeling was utilized for hypothesis testing. ADs reported significantly higher

sedation and craving at the start of the alcohol infusion and exhibited a blunted response along escalating BrAC. ADs also exhibited greater tension at the start of the infusion but did not differ in tension reduction from HDs. Furthermore, stimulation was associated with alcohol craving to a significantly greater extent in HDs as compared to ADs ($\beta = 0.718$, 0.169 respectively, difference $p < 0.05$). Furthermore, tension at the start of the alcohol infusion was positively associated with craving in ADs ($\beta = 0.386$, $p < 0.05$), but not in HDs ($\beta = 0.004$, $p > 0.10$).This study extends the literature by demonstrating that HDs and ADs differ in their subjective experience of alcohol as well as in the association between SR and craving for alcohol. Hypotheses derived from Koob's allostatic model of alcoholism etiology were partially supported. Critically, analysis revealed that, while ADs and HDs did not differ in the magnitude of positive hedonic response to alcohol, there was a relative disconnect between subjective reward and craving in ADs as compared to HDs. These results are consistent with the notion that acute reward from alcohol is more salient as a determinant of craving (and of drinking) during sub-clinical heavy drinking as compared to during clinically significant alcohol dependence.

Disclosures: S. Bujarski: None. **J. Jentsch:** None. **L.A. Ray:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.14/JJ6

Topic: C.18. Drugs of Abuse and Addiction

Support: Pearson Center for Alcoholism and Addiction Research

NIH AA008459

NIH AA006420

Title: Increased emission of rat ultrasonic vocalization (USV) over the course of alcohol dependence

Authors: *C. L. BUCK[1,2], J. E. SCHLOSBURG[2], L. J. LIPPERT[1], G. F. KOOB[2], L. F. VENDRUSCOLO[2];
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Abstract: Rats made dependent on alcohol by chronic, intermittent alcohol vapor exposure exhibit increased alcohol self-administration and compulsive-like alcohol intake compared with

nondependent rats. We have found that alcohol dependent rats display increased emission of 50 kHz USVs, which are associated with positive affective-like states, during anticipation of alcohol self-administration, and increased stress-induced 22 kHz USVs, an index of negative affective-like states, during acute alcohol withdrawal, compared with nondependent rats. We tested the hypothesis that anticipatory 50 kHz and 22 kHz USVs would follow a pattern consistent with escalation of alcohol self-administration during the development of alcohol dependence. Over the course of vapor exposure, we observed that rats displayed a transient increase in anticipatory 50 kHz USVs, whereas stress-induced 22 kHz USVs exhibited a more sustained increase. Additionally, the benzodiazepine chlordiazepoxide, a class of drugs used for alcohol detoxification, decreased stress-induced 22 kHz USVs. Together, these findings suggest that 50 kHz and 22 kHz USVs represent distinct behavioral indicators of increased motivation for alcohol self-administration during alcohol dependence and can help to dissect the neurobiological mechanisms of compulsive-like alcohol drinking. Ongoing studies are investigating the effects of glucocorticoid receptor antagonism, which has been reported to decrease compulsive-like alcohol drinking, on 22 kHz USVs during acute withdrawal in dependent and nondependent rats.

Disclosures: C.L. Buck: None. **L.F. Vendruscolo:** None. **J.E. Schlosburg:** None. **G.F. Koob:** None. **L.J. Lippert:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.15/JJ7

Topic: C.18. Drugs of Abuse and Addiction

Support: NIAAA U01 AA020929

NIAAA U01 AA014095

Title: Voluntary ethanol intake in ethanol-dependent and nondependent NR2B conditional KO mice

Authors: *M. F. LOPEZ[1], R. L. MCCANN[1], J. L. HOPKINS[1], M. P. OVERSTREET[1], E. DELPIRE[2], P. J. MULHOLLAND[1], H. C. BECKER[1];
[1]Psychiatry, Med. Univ. of South Carolina, CHARLESTON, SC; [2]Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Repeated cycles of chronic intermittent ethanol (CIE) exposure produces escalation of voluntary ethanol drinking in C57BL/6J mice. Given the role of glutamate/NMDA receptor activity in ethanol dependence, this study evaluated the effect of CIE exposure in conditional NR2B KO mice. Conditional NR2B KO mice were generated by crossing homozygous-floxed mice carrying CAMKIIa-tTA and mice with the tetO-driven CRE transgene while breeding pairs were maintained on a doxycycline containing diet. Deletion of the NR2B gene was induced by removing the doxycycline diet at 45 days of age, and evidence for reduced expression was verified in cortex and striatum by Western blots at 75 and 200 (but not 45) days of age in KO mice. At adulthood, mice were tested for voluntary ethanol intake using a limited access (2 hr/day) procedure, with the ethanol concentration gradually increased from 3 to 15% (v/v) over several days. During this period, female NR2B KO mice showed higher levels of intake compared to female WT controls. Male KO and WT mice had a similar level of intake. Once stable baseline intake of 15% ethanol was observed, NR2B KO and WT mice from both sexes received 4 weekly cycles CIE vapor exposure (EtOH group) or air exposure (CTL group) (16 hr/day x 4 days) alternated by 5-day drinking test cycles (C57BL/6J male mice were included as positive controls). As expected, CIE exposure induced a significant increase in voluntary ethanol intake in C57BL/6J male mice. Ethanol intake increased from 2 g/kg at baseline to 3 g/kg during Test 4 in the EtOH group, while intake remained stable throughout all test periods in CTL mice. Although, NR2B KO and WT males had a lower baseline intake level than C57BL/6J mice (~1.2 g/kg), CIE exposure also induced a significant increase in voluntary ethanol intake in these mice. In females, CIE exposure induced higher voluntary ethanol intake only in KO mice. Further evaluation of these mice indicated that female NR2B KO mice showed higher levels of locomotor activity in an open field compared to female WT and male KO and WT. Female KO mice also failed to adapt (continued to struggle and swim) to repeated daily testing in the Porsolt forced swim test. Overall, these results indicate that deletion of the NR2B receptor subunit influences locomotor activity, adaptation to repeated stress, and voluntary ethanol intake in ethanol dependent and nondependent, with more robust effects observed in female mice.

Disclosures: M.F. Lopez: None. **R.L. McCann:** None. **J.L. Hopkins:** None. **M.P. Overstreet:** None. **E. Delpire:** None. **P.J. Mulholland:** None. **H.C. Becker:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.16/JJ8

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant R01AA019458

Title: Ceftriaxone-induced upregulation of GLT-1 isoforms and xCT attenuates in part relapse-like ethanol-drinking behavior in male alcohol-preferring (P) rats

Authors: H. ALHADDAD, *Y. SARI;
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Abstract: Studies from our laboratory demonstrated that ceftriaxone-induced upregulation of glutamate transporter 1 (GLT-1) in prefrontal cortex (PFC) and nucleus accumbens (NAc) reduced ethanol intake after five weeks of free-choice ethanol drinking paradigm in male alcohol-preferring (P) rats. GLT-1 exists in two splice variant isoforms: GLT-1a and GLT-1b. Of these variants, GLT-1a is predominantly in astrocytes and neurons, and GLT-1b is mainly expressed in astrocytes. In this study, we investigated the effect of ceftriaxone on the levels of GLT-1 isoforms and other glial cell proteins such as glutamate aspartate transporter (GLAST) and cysteine/glutamate exchanger (xCT) in relapse-like ethanol-drinking behavior. P rats were exposed to free choice of 15% and 30% ethanol and to water for five weeks. Then they were treated with ceftriaxone (100 mg/kg, i.p.) or saline vehicle during the last five days of the two-week deprivation period. We found that ceftriaxone treatment induced significant attenuation in relapse-like ethanol-drinking behavior that persisted for nine days upon re-exposure to ethanol. However, water intake increased significantly in ceftriaxone-treated rats compared to saline-treated rats. Importantly, ceftriaxone-mediated attenuation in relapse-like ethanol-drinking behavior was associated with upregulation of the levels of GLT-1a and GLT-1b isoforms, and xCT in PFC and NAc. We did not observe any significant differences in GLAST expression among all groups, which revealed the specific action of ceftriaxone in GLT-1 isoforms and xCT expression. Ceftriaxone-induced upregulation of GLT-1 isoforms and xCT, associated in part with the attenuation of relapse-like ethanol-drinking behavior, may suggest the potential therapeutic uses of this drug in the treatment of relapse to alcohol consumption.

Disclosures: H. Alhaddad: None. **Y. Sari:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.17/JJ9

Topic: C.18. Drugs of Abuse and Addiction

Support: CAPES

Title: Differential patterns of expression of Neuropeptide Y throughout withdrawal in outbreed Swiss mice classified as susceptible or resistant to locomotor sensitization induced by ethanol

Authors: *L. S. COELHO[1], R. DE PAULI[3], N. F. CORREA-NETTO[2], J. G. SANTOS-JUNIOR[2];
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Abstract: Several studies in drug addiction have focused on the negative emotional state emerging from withdrawal. In this scenario NPY plays an important role, given its involvement in drug addiction, anxiety and mood disorders. Locomotor sensitization is a useful animal model to investigate the neuronal plasticity induced by drugs of abuse and there is an important behavioral variability in outbreed Swiss mice to develop ethanol induced locomotor sensitization. We investigated whether this variability is also applied to the NPY expression during withdrawal. Outbreed Swiss mice were daily treated with ethanol (or saline) for 21 days. According to locomotor activity after last injection, ethanol group was classified as sensitized (EtOH_High) or non-sensitized (EtOH_Low). Furthermore, after 5 days of withdrawal, some animals were challenged with ethanol. To evaluate NPY expression, mice were sacrificed at 18 hours or 5 days of withdrawal, as well as 1.5 hours after challenge. At 5 days of withdrawal, NPY increased in the orbital cortex, dorsomedial striatum, piriform cortex and dentate gyrus of EtOH_High group. These changes were counteracted by ethanol challenge. In EtOH_Low group, increases on NPY in the dentate gyrus occurred early (18 h of withdrawal). Ethanol challenge also decreased (EtOH_High) and increased (EtOH_Low) NPY in piriform cortex. Finally, NPY decreased in the prelimbic cortex of EtOH_Low group at 5 days of withdrawal, and this decrease was reversed by ethanol challenge. In conclusion, these results suggest that behavioral variability in the locomotor sensitization seems to be useful to study neurobiological mechanisms of ethanol withdrawal. Furthermore it is possible that the changes on NPY expression during withdrawal could play both adaptive and pathological consequences. For example, in mice susceptible to locomotor senstitization, increased NPY in striatum and orbitofrontal cortex could be considered as an allostatic mechanism, while in the piriform cortex and dentate gyrus a homeostatic mechanism. Moreover, similar homeostatic features could be hypothesized for the decreased NPY expression in the prelimbic cortex of mice that did not develop locomotor sensitization. Therefore, besides the well established effects of chronic drug exposure over NPY expression, withdrawal also plays a pivotal role determining expressive changes in NPY expression.

Disclosures: L.S. Coelho: None. **R. De Pauli:** None. **N.F. Correa-Netto:** None. **J.G. Santos-Junior:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.18/JJ10

Topic: C.18. Drugs of Abuse and Addiction

Support: NIAAA RO1AA016959

NIAAA F31AA019853

NIAAA RO1AA013388

NIAAA RO1AA013561

Title: Cannabinoid receptor 1 activation selectively inhibits ethanol withdrawal induced potentiation of NMDA neurotoxicity

Authors: *D. J. LIPUT, M. A. PRENDERGAST, K. NIXON;
Univ. of Kentucky, Lexington, KY

Abstract: Excessive alcohol intake, characteristic of alcohol use disorders (AUDs), results in neurodegeneration which is theorized to influence the transition to addiction. Therefore, it is hypothesized that neuroprotective agents will have utility for the treatment of AUDs. The endocannabinoid (eCB) system has emerged as a potent neuroprotective target for a wide range of neurodegenerative disorders; however few reports have investigated the eCB system in models of alcohol induced neurodegeneration. Therefore, the current study investigated the effect of cannabinoid 1 receptor (CB1R) activation on withdrawal-induced excitotoxicity in an organotypic hippocampal slice culture (OHSC) model. OHSCs were prepared from 8 day old Sprague-Dawley rat pups and were matured ex vivo for 5 days before drug treatments. At 6 days, slices were exposed to either 50 mM ethanol (EtOH) or control media for 10 days. Following EtOH exposure, slices underwent a 24 hr EtOH withdrawal (EWD) in the presence of different drug combinations and propidium iodide (PI, 2.5 μg/mL) to assess toxicity. Following withdrawal, cultures were imaged for PI uptake using fluorescence microscopy and analyzed for optical density in CA1, CA3 and the dentate gyrus (DG) regions of the hippocampus. As shown previously, ethanol exposure potentiated NMDA (5μM) toxicity in CA1 ($p < 0.001$), while toxicity was not observed in CA3 or the dentate gyrus (DG) in either NMDA or EWD+NMDA cultures. Application of the CB1R agonist, CP55940, dose dependently blocked EWD potentiation of NMDA toxicity in CA1 compared to EWD+NMDA treated cultures, which was significant at 1.0 μM ($p < 0.05$) and 10.0 μM ($p < 0.01$). Furthermore, co-application of the CB1R antagonist SR141716 (10.0 μM) completely abrogated CP55940 mediated neuroprotection ($p < 0.01$). Interestingly, neuroprotection was specific to toxicity associated with EWD as CP55940 failed to block toxicity following NMDA application alone. In contrast to CP55940, the FAAH inhibitor URB597 (50 nM - 500 nM) failed to block EWD potentiation of NMDA toxicity in preliminary experiments. The current study found that the eCB system is a

viable target for preventing ethanol withdrawal induced neurotoxicity. As CB1 agonism has low therapeutic utility, due to an unfavorable side effect profile, future studies will evaluate other strategies to target the eCB system, such as blocking 2-AG catabolism and duel blockage of AEA and 2-AG catabolism. In conclusion, the eCB system may be a viable target to prevent neurotoxicity associated with alcohol withdrawal. Funded by NIAAA R01AA016959 (KN), F31AA019853 (DL), R01AA013388 (MP) and R01AA018588 (MP).

Disclosures: D.J. Liput: None. **M.A. Prendergast:** None. **K. Nixon:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.19/JJ11

Topic: C.18. Drugs of Abuse and Addiction

Title: The role of beta1 adrenoceptors in the development of alcohol dependence

Authors: *P. M. KLENOWSKI[1], J. HOLGATE[1], M. BELLINGHAM[2], P. MOLENAAR[1], S. BARTLETT[1];
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Abstract: There is significant evidence supporting the hypothesis that stressors play an important role in the development of alcohol dependence. Many investigators are dissecting the molecular mechanisms that underpin the role of stress in the development of alcohol dependence to improve upon the current pharmotherapeutics. Compounds that block norepinephrine receptors such as alpha1-adrenergic receptors using prazosin (Walker et al., 2008) or beta-adrenoceptor using propranolol have all shown promise as potential therapeutic agents (Gilpin and Koob, 2010). We have extended these studies to characterize the role of the beta1-adrenoceptor (AR) in ethanol consumption using the drinking in the dark (DID) protocol in mice and using to radioligand binding experiments to characterize the receptor in the brain of naïve and ethanol consuming mice. Briefly, mice were housed individually in a reverse light-dark cycle room and given access to 1 bottle of 20% ethanol (v/v) and 1 bottle of filtered water for a 2 hour period, 5 days a week, 3 hours into the dark cycle. Bottles were weighed 30 min and 2 hours after presentation to determine daily ethanol consumption. The beta1-adrenoceptor contains two binding sites, beta1H and beta1L, corresponding to high and low-affinity binding sites respectively. Receptor activation can occur through both binding sites. Some beta-blockers typified by (-)-CGP 12177 block beta1AR and beta2ARs, but can also activate beta1LARs at higher concentrations than those required to cause blockade (Kaumann and Molenaar, 2008).

The role of beta1LARs have been extensively characterized in the heart. The mammalian brain is also an area of high beta1AR expression, however it is unknown whether beta1LARs exist in this region nor its role in stress and ethanol consumption. We show that there is high beta1AR expression in mouse brain using radioligand binding studies with (-)-[3H]-CGP 12177 to label beta1AR binding sites in brains from C57BL/6 mice. We then show that (-)-CGP 12177 reduces ethanol consumption in mice consuming ethanol using the DID protocol. We are currently screening a series of compounds with activity at beta1HAR and beta1LARs to determine their effect on ethanol consumption using an adapted drinking-in-the-dark paradigm established in our lab. Of particular interest is our data showing that the beta1LAR partial agonist (-)-CGP 12177 decreases ethanol consumption in C57BL/6 mice. Activation of the beta1AR through the beta1L site may represent a novel therapeutic strategy for the management of alcohol addiction.

Disclosures: P.M. Klenowski: None. **J. Holgate:** None. **M. Bellingham:** None. **P. Molenaar:** None. **S. Bartlett:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.20/JJ12

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant AA017371

NIH Grant AA020960

NIH Grant AA013191

NIH Grant AA013523

Title: MeCP2 regulates ethanol sensitivity and intake

Authors: *J. CHEN[1], V. REPUNTE-CANONIGO[1], C. LEFEBVRE[2], T. KAWAMURA[1], M. KREIFELDT[1], O. BASSON[1], A. ROBERTS[1], P. P. SANNA[1];
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Abstract: We have investigated the expression of chromatin-regulating genes in the prefrontal cortex and in the shell subdivision of the nucleus accumbens during protracted withdrawal in mice with increased ethanol drinking after chronic intermittent ethanol (CIE) vapor exposure and in mice with a history of non-dependent drinking. We observed that the methyl-CpG binding protein 2 (MeCP2) was one of the few chromatin-regulating genes to be differentially regulated

by a history of dependence. As MeCP2 has the potential of acting as a broad gene regulator, we investigated sensitivity to ethanol and ethanol drinking in MeCP2308/Y mice, which harbor a truncated MeCP2 allele but have a milder phenotype than MeCP2 null mice. We observed that MeCP2308/Y mice were more sensitive to ethanol's stimulatory and sedative effects than wild-type (WT) mice, drank less ethanol in a limited access 2 bottle choice paradigm and did not show increased drinking after induction of dependence with exposure to CIE vapors. Alcohol metabolism did not differ in MeCP2308/Y and WT mice. Additionally, MeCP2308/Y mice did not differ from WT mice in ethanol preference in a 24-hour paradigm nor in their intake of graded solutions of saccharin or quinine, suggesting that the MeCP2308/Y mutation did not alter taste function. Lastly, using the Gene Set Enrichment Analysis algorithm, we found a significant overlap in the genes regulated by alcohol and by MeCP2. Together, these results suggest that MeCP2 contributes to the regulation of ethanol sensitivity and drinking.

Disclosures: J. Chen: None. **V. Repunte-Canonigo:** None. **C. Lefebvre:** None. **T. Kawamura:** None. **M. Kreifeldt:** None. **O. Basson:** None. **A. Roberts:** None. **P.P. Sanna:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.21/JJ13

Topic: C.18. Drugs of Abuse and Addiction

Title: Ethanol-induced epigenetic modifications mediate behavioral plasticity

Authors: *G. L. ENGEL[1], B. M. ZIMAN[1], K. R. KAUN[2], S. MARELLA[3], E. C. KONG[3], F. W. WOLF[1];
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Abstract: Alcohol is the most widely abused drug, with high social and economic costs to humanity. Despite this, the mechanisms by which alcohol affects brain function and behavior are not yet fully understood. Epigenetic regulation of transcription may be a key mechanism for regulating the behavioral plasticity induced by exposure to drugs of abuse. Our lab uses a rapid ethanol tolerance paradigm in Drosophila melanogaster to study the cellular and molecular mechanisms of ethanol-induced behavioral plasticity. Using this model we discovered that a highly conserved histone/protein deacetylase (HDAC) plays a critical role in the behavioral

response of the flies to ethanol. In HDAC mutant adults there is a decrease in both the development of tolerance and of reward, two behavioral responses to ethanol that play an important role in its addictive properties. These behavioral deficits can be rescued by restoring HDAC expression in neurons, and can be phenocopied by decreasing brain HDAC expression in wild-type flies. Further, HDAC expression is reduced and specific histone modifications are increased in an HDAC-dependent manner following ethanol exposure. Combined, these data indicate an important role for an HDAC in the orchestration of ethanol behaviors and suggest an epigenetic mechanism for the induction of behavioral plasticity in response to drugs of abuse.

Disclosures: G.L. Engel: None. **B.M. Ziman:** None. **F.W. Wolf:** None. **S. Marella:** None. **E.C. Kong:** None. **K.R. Kaun:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.22/JJ14

Topic: C.18. Drugs of Abuse and Addiction

Support: NIAAA Grant F32 AA020430

NIAAA Grant AA020913

NIAAA Grant AA006420

NIAAA Grant AA013498

NIAAA Grant AA015566

Title: Cell type-specific alterations in tonic GABAA receptor transmission in the central amygdala of CRF receptor-1 reporter mice following chronic ethanol exposure

Authors: *M. A. HERMAN, C. CONTET, M. ROBERTO;
CNAD, The Scripps Res. Inst., La Jolla, CA

Abstract: We have previously shown that there is cell-type specific tonic GABAA receptor conductance in the central amygdala (CeA) of a CRF receptor-1 reporter mouse model. Specifically, neurons containing the CRF1 receptor (CRF1+) are composed of both the low threshold bursting (LTB) and regular spiking (RS) cell types and display a significant baseline tonic conductance that is action-potential dependent and insensitive to acute ethanol. Neurons lacking the CRF1 receptor (CRF1-) are composed of RS and late spiking (LS) cell types and do

not display baseline tonic conductance but do possess the potential for a tonic conductance that is enhanced by acute ethanol. The present study was undertaken to examine changes in baseline tonic conductance following chronic intermittent ethanol (CIE) exposure. CRF1:GFP mice were subjected to 4-5 weeks of CIE in ethanol inhalation chambers or air/pyrazole control treatment and whole cell voltage-clamp recordings were performed in CRF1+ and CRF1- CeA neurons immediately following exposure or 5-7 days into withdrawal. Consistent with what was observed in naïve mice, CRF1+ neurons of both LTB and RS cell types from control mice displayed a significant tonic conductance following focal application of 100 μM gabazine (LTB: 14.2 ± 2.1 pA, n = 8; RS: 17.0 ± 2.9 pA, n = 5). However, this tonic conductance was not observed in LTB or RS CRF1+ neurons from CIE-treated mice immediately following ethanol exposure or 5-7 days into withdrawal. Conversely, CRF1- CeA neurons of both LS and RS cell types from control mice did not display a baseline tonic conductance, but a significant tonic conductance was observed following focal application of 100 μM gabazine in LS CRF1- neurons from CIE-treated mice immediately following ethanol exposure (11.1 ± 1.3 pA, n = 6) and 5-7 days into withdrawal (14.5 ± 2.7 pA, n = 10). No change in tonic conductance was observed in RS CRF1- neurons, suggesting that the upregulation of tonic conductance by CIE occurs only in a specific subpopulation of CRF1- CeA neurons. Consistent with this hypothesis, RS CRF1- neurons displayed a significantly lower sensitivity to the δ-subunit preferring GABAA receptor agonist THIP than LS CRF1- neurons in control mice. Collectively these data demonstrate that cell-type specific alterations in tonic GABAA receptor signaling occur in the CeA following chronic ethanol exposure. These alterations likely have significant effects on the overall activity of the CeA and this disrupted activity may contribute to cycle of negative reinforcement that leads to the behavioral manifestation of alcohol dependence.

Disclosures: M.A. Herman: None. **C. Contet:** None. **M. Roberto:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.23/JJ15

Topic: C.18. Drugs of Abuse and Addiction

Support: ERA-Net TRANSALC 01EW1112

Center grant SFB636 B1

Title: Distinct metabolic alterations in reward related brain areas of rats with a history of alcohol dependence

Authors: *M. MEINHARDT[1], D. C. SÉVIN[2], M. L. KLEE[1], S. DIETER[1], U. SAUER[2], W. H. SOMMER[1];
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Abstract: Recently, we demonstrated the critical importance of frontostriatal circuits for the expression of addiction like behavior in a rat model of alcoholism. Specifically, the infralimbic cortex seemed to be a highly sensitive target for alcohol-induced pathology. However, the underlying neurobiology of these region-specific changes is poorly understood. Mass-spectroscopy allows assessing global metabolic profiles in specific brain regions, and thus provides a tool to search for novel pathophysiological mechanisms.
Here we tested the hypothesis that a history of alcohol dependence manifest itself in an altered cerebro-metabolic phenotype in frontostriatal brain regions. We induced alcohol dependence in rats in using intermittent alcohol vapor exposure for seven weeks. Following three weeks of abstinence, rats had access to alcohol (8% v/v) and tap water for seven weeks, before two frontal (infralimbic and prelimbic) and two striatal (nucleus accumbens core and shell) brain areas were micro-dissected for a global metabolic screening.
2D-Principal component loadings of the dataset displayed that the infra- and prelimbic cortex have a metabolic phenotype that is distinct from the two accumbal regions, which do not separate. Among the top 50 compounds explaining most of the variance to separate the brain regions, are known neuroactive compounds such as N-Acetylaspartate, glutamate, glutamine, creatinine, GABA, myo-Inositol and dopamine. Next, with the use of a metabolic pathway enrichment analysis, we found the valine, leucine and isoleucine biosynthesis pathways in the cortical as well as the nicotinate and nicotinamide metabolism is highly enriched in accumbal structures. Finally, with a targeted analysis, the intensities of several compounds known to be involved in addiction were extracted from the data set and compared with control rats. We found several metabolites significantly altered in both cortical regions, including GABA, MET-enkephalin and dopamine. Strikingly, dopamine concentrations highly correlated with the amount of alcohol consumed in the two bottle-free-choice paradigm.
Together, these data point towards profound alterations in all analyzed brain structures, in particular within the cortical regions. In addition, we could show that two anatomical similar structures as the pre- and infralimbic cortex express a completely different metabolic phenotype, which has to be taken into consideration for future studies in the field of neurophysiological investigations.

Disclosures: M. Meinhardt: None. **M.L. Klee:** None. **S. Dieter:** None. **W.H. Sommer:** None. **D.C. Sévin:** None. **U. Sauer:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.24/JJ16

Topic: C.18. Drugs of Abuse and Addiction

Support: Regione Autonoma della Sardegna (RAS), Progetti di ricerca di base - Bando 2008, to M.D.

Title: Profound and selective decrease of dendritic spines in the nucleus accumbens of ethanol dependent rats

Authors: *S. SPIGA[1], G. MULAS[1], G. MUGGIRONI[2], G. FOIS[2], C. CANNIZZARO[4], M. DIANA[3], M. DIANA[3];
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Abstract: Neuronal refinement and stabilization are hypothesized to confer resilience to poor decision-making and addictive-like behaviors, such as excessive ethanol drinking and dependence. Accordingly, structural abnormalities are likely to contribute to the appearance of alcohol withdrawal signs and symptoms, that occur from suddenly ceasing the use of alcohol after chronic ingestion, thus perpetuating the addictive cycle. Here we show that ethanol dependent rats display a loss of dendritic spines in medium spiny neurons of the Nacc, accompanied by a reduction of TH-positive terminals and PSD-95 positive elements. Further analysis indicates that 'long thin', but not 'mushroom', spines are selectively affected. These changes are restricted to the withdrawal phase of ethanol dependence suggesting their relevance in the genesis of signs and/or symptoms affecting ethanol withdrawal, and thus the Whole addicting cycle. Overall these results highlight the importance of spine function on the evolution of alcohol dependence and suggest that the selective loss of 'long thin' spines may affect learning dysfunctions and significantly contribute to further 'impoverish' the already deficient dopaminergic transmission whose hypofunctionality is a major factor for the emergence of the harmful consequences of alcohol abuse/dependence.

Disclosures: S. Spiga: None. **G. Mulas:** None. **G. Muggironi:** None. **G. Fois:** None. **C. Cannizzaro:** None. **M. Diana:** None. **M. Diana:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.25/JJ17

Topic: C.18. Drugs of Abuse and Addiction

Support: 1F31AA022032-01

AA019967

AA010983

AA017922

Title: Homeostatic changes in NMDA receptors and Kv4.2 channels following chronic ethanol exposure is accompanied by alterations in FMRP phosphorylation

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Abstract: Exposure to chronic ethanol results in significant, long-term adaptations at glutamatergic synpases that alter signal transduction and cellular responses. Specifically, prolonged ethanol exposure increases synaptically expressed NMDA receptor protein levels as well as NMDA receptor activity. However, the mechanism by which these homeostatic changes occur is unknown. In previous studies, the mRNA-binding protein fragile-X mental retardation protein (FMRP) alters both NMDA receptors and the voltage-dependent Kv4.2 channel in an activity-dependent manner. Increases in FMRP phosphorylation stimulate binding to Kv4.2 mRNA and thus decrease translation. In organotypic hippocampal slice cultures, we examined the effect of NMDA receptor activity on FMRP phosphorylation. Exposure of 100 μM NMDA for 5, 15, and 30 minutes reduced FMRP phosphorylation, but did not alter total protein levels. Incubating slice culture media with the NMDA receptor antagonist AP-V altered FMRP phosphorylation levels in a dose-dependent manner. Because chronic and acute ethanol exposure have previously been shown to alter NMDA receptor expression and activity, we next examined the effect of 75 mM ethanol exposure on total FMRP protein levels as well as changes in FMRP phosphorylation. Twenty-four hour exposure to ethanol increased phosphorylated FMRP while total protein levels remained unchanged. This increase in phosphorylation without a change in total protein persisted following a chronic (8-day) exposure to ethanol. We also observed a decrease in Kv4.2 surface expression that is mirrored by an increase in NMDA receptor expression following this 8-day ethanol exposure paradigm. Additionally, the hyperexcitability initiated by the removal of ethanol from the culture media is accompanied by a decrease in FMRP phosphorylation. These data are consistent with the model that FMRP phosphorylation may influence NMDA receptor activity-dependent changes in Kv4.2 following chronic ethanol exposure.

Disclosures: K. Spencer: None. **P. Mulholland:** None. **L. Chandler:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.26/JJ18

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH grant AA020913 (CC)

NIH grant AA020893 (AJR)

NIH grant AA013517 (GFK)

Title: BK channel beta1 and beta4 subunits play differential roles in the physical and motivational effects of ethanol withdrawal

Authors: *C. CONTET[1], D. LE[1], M. KREIFELDT[1], S. N. TREISTMAN[2], A. J. ROBERTS[1], G. F. KOOB[1];
[1]Scripps Resch Inst., LA JOLLA, CA; [2]Inst. of Neurobio., Univ. of Puerto Rico, San Juan, PR

Abstract: Alcohol dependence has devastating health and societal consequences. One of the well-established molecular targets of ethanol is the large conductance calcium-activated potassium (BK) channel. BK channels play a key role in several aspects of neuronal physiology. Ethanol is a potent activator of BK channel gating, but in vivo evidence for a causal relationship between BK channel potentiation and ethanol's behavioral effects is scarce. Interestingly, association of the auxiliary beta1 or beta4 subunit with the pore-forming alpha subunit reduces ethanol-induced potentiation of BK currents in vitro. In the present study, we investigated whether BK beta1 and beta4 subunits influence the physical and motivational effects of ethanol withdrawal using knockout mice. Handling-induced convulsions (HIC) were assessed following i.p. injection of 4 g/kg ethanol, before and after exposure to chronic intermittent ethanol (CIE) in inhalation chambers. Owing to their C57Bl/6J background, wildtype mice exhibited minimal physical withdrawal under both conditions. Deletion of BK beta4 subunit significantly increased HIC severity both in naïve and CIE-exposed mice. BK beta1 knockout mice did not differ from their wildtype littermates pre-CIE, but displayed HIC earlier in withdrawal post-CIE. To assess the motivational aspect of withdrawal, independent cohorts of mice were first trained to drink ethanol in a limited-access two-bottle choice (2BC) paradigm. BK beta1 or beta4 deletion did not affect baseline levels of drinking. Weeks of 2BC were then alternated with weeks of CIE (dependent mice) or air (non-dependent mice) exposure. A gradual escalation of voluntary

ethanol intake was observed in dependent wildtype mice, while intake remained stable in non-dependent wildtype mice. In contrast, CIE exposure did not alter the ethanol consumption of BK beta4 knockout mice. Conversely, ethanol drinking increased after fewer CIE cycles in BK beta1 knockout mice than in wildtype mice. In conclusion, absence of either BK beta1 or beta4 exacerbates physical withdrawal from ethanol, but deletion of BK beta4 prevents, while deletion of BK beta1 accelerates, the escalation of ethanol drinking during withdrawal from CIE. These results highlight the functional dissociation between mechanisms mediating the physical and motivational effects of ethanol withdrawal. In addition, our data suggest that BK beta1 and beta4 have an opposite influence on the negative reinforcing properties of ethanol withdrawal. Modulating the expression, cellular distribution or interaction properties of BK channel auxiliary subunits may represent a novel avenue for the treatment of alcoholism.

Disclosures: C. Contet: None. **D. Le:** None. **M. Kreifeldt:** None. **S.N. Treistman:** None. **A.J. Roberts:** None. **G.F. Koob:** F. Consulting Fees (e.g., advisory boards); Addex Pharmaceuticals, Arkeo Pharmaceuticals, Embera NeuroTherapeutics, Psychogenics.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.27/KK1

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH grant T32AA007468

NIH grant AA016981

NIH grant AA016647

Title: Differential effects of ghrelin antagonists on alcohol drinking following chronic intermittent ethanol vapor exposure in mice

Authors: *J. L. GOMEZ, C. SNELLING, D. A. FINN, A. E. RYABININ;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Alcohol addiction is a medical disease that causes both personal problems and takes a vast toll on society. It is imperative to find treatments that suppress alcohol drinking in individuals who suffer from this disorder. Recent research has shown that ghrelin receptor antagonists have the ability to suppress drinking and alleviate some neurological changes caused by excessive alcohol consumption. In this study, we used 3 days of chronic intermittent ethanol

vapor exposure (CIE), which is an established technique for increasing alcohol consumption in dependent C57BL/6 mice. We tested whether two different ghrelin receptor antagonists (DLys3-Ghrp-6 and JMV2959) would suppress the increase in ethanol consumption following CIE. Male C57BL/6 mice were randomly assigned to six groups based on drug and vapor exposure: Vehicle-Air, Vehicle-CIE, JMV-Air, JMV-CIE, DLys-Air, and DLys-CIE. Using a 2-bottle choice drinking-in-the-dark procedure, baseline drinking of 15% v/v ethanol was established before any drug or vapor exposure, with no statistical differences found between pre-assigned groups. Following both the first and second cycles of 3 days of intermittent vapor exposure, we found that both ghrelin antagonists suppressed ethanol consumption regardless of vapor exposure; however, the effects were transient. On day-1, ethanol intake after the first vapor or air exposure was suppressed by JMV and DLys. Intake returned to vehicle levels by day-3 in the DLys group, but the JMV group continued to show reduced intake. After the second vapor or air exposure, the drug effects were the same, with JMV and DLys suppressing intake. However, by day-2 (DLys) and day-3 (JMV) the drug groups returned to control levels of drinking. The preferences and total fluid intake data during the 2-hour ethanol exposure overall reflected the measured ethanol intake. However, the DLys treated mice showed a decrease in preference and the JMV mice showed a reduced total fluid intake. Thus, the drugs appear to differ in specificity of their actions on ethanol intake. It is possible that this difference reflects previous findings indicating that JMV has more pharmacologically specific effects on ghrelin receptors than DLys. It should be noted that over a 24-hour period, water and food consumption was not different between groups. In conclusion, while ghrelin antagonists might be adequate at suppressing increased ethanol intake by exposure to CIE, the effects were transient. Therefore, future research will attempt to reduce ghrelin receptor expression in specific regions on a longer term basis to determine if targeting this receptor can be used for alcohol abuse and dependence treatment.

Disclosures: J.L. Gomez: None. **C. Snelling:** None. **D.A. Finn:** None. **A.E. Ryabinin:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.28/KK2

Topic: C.18. Drugs of Abuse and Addiction

Support: HA 6102/1-1

ERA-Net TRANSALC 01EW1112

DFG SFB636

Title: Dopamine system adaptations in alcohol abstinence: Evidence from humans and rats for a hyperdopaminergic state

Authors: *N. HIRTH[1], M. W. MEINHARDT[1], L. BROCCOLI[1], S. PERREAU-LENZ[1], S. UHRIG[1], R. RIMONDINI[2], C. HARPER[3], M. HEILIG[4], R. SPANAGEL[1], W. H. SOMMER[1], A. C. HANSSON[1];
[1]Psychopharmacology, Central Inst. of Mental Hlth., Mannheim, Germany; [2]Univ. of Bologna, Bologna, Italy; [3]The Univ. of Sydney, Sydney, Australia; [4]Natl. Inst. on Alcohol Abuse and Alcoholism (NIAAA), Bethesda, MD

Abstract: The mesocorticolimbic dopamine (DA) system is critically involved in addiction and undergoes pronounced neuroadaptations after chronic alcohol consumption. Acute withdrawal from alcohol has consistently been shown to down-regulate DAergic neurotransmission while reports on the state of the mesocorticolimbic DA system in abstinence are contradictory. Human positron emission tomography studies on abstinent alcoholics suggest mostly attenuated striatal DRD2 and DA levels. The DA receptor D1 (DRD1) has so far been less studied.
Here, we investigated the expression of DRD1 and DRD2 as well as the DA transporter (DAT) on both the mRNA and the protein level using post-mortem striatal brain tissue of human male alcoholic and control subjects by qRT-PCR and receptor autoradiography. To gain further insight into underlying mechanisms we compare these data to three weeks abstinent rats in which alcohol dependence was induced by chronic intermittent alcohol vapor exposure. In these animals, we analyzed expression of DRD1, DRD2, DAT and tyrosine hydroxylase (TH) by in situ hybridization and receptor autoradiography. Extracellular DA levels were measured within the nucleus accumbens of PD rats and controls by in vivo microdialysis.
We found highly significant down-regulation of DRD1 and DAT binding in the ventral striatum and nucleus caudatus of human alcoholics, while DRD2 was unaffected. Also in alcohol abstinent rats we found reduced striatal DRD1 levels as well as increased mRNA expression of the DA synthesizing enzyme TH in the substantia nigra pars compacta. Together these data point to a hyperdopaminergic state during abstinence from alcohol dependence. This hypothesis was confirmed by in vivo microdialysis showing increased extracellular DA levels in the Acb shell region of abstinent rats.
Our data indicate increased DA levels during alcohol abstinence and support the hypothesis of a hyperdopaminergic state in abstinent alcoholics that may exist at least in a subgroup of alcoholics. Future studies are needed to reveal the mechanisms underlying the altered DA transmission in alcohol dependence.

Disclosures: N. Hirth: None. **M.W. Meinhardt:** None. **L. Broccoli:** None. **S. Perreau-Lenz:** None. **S. Uhrig:** None. **R. Rimondini:** None. **C. Harper:** None. **M. Heilig:** None. **R. Spanagel:** None. **W.H. Sommer:** None. **A.C. Hansson:** None.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.29/KK3

Topic: C.18. Drugs of Abuse and Addiction

Support: This study was supported by D&A Pharma

Title: Pharmacodynamic interactions of a solid formulation of sodium oxybate and alcohol in healthy volunteers

Authors: *N. PROSS[1], N. FAUCHOUX[2], H. HADJDUCHOVA[3], C. DENOT[2], A. DUFOUR[4], A. PATAT[2], P. VIVET[3];
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Abstract: *Introduction:* A liquid formulation of Sodium oxybate (SO) has been approved for the treatment of alcohol dependence (AD) in Europe. The pharmacological effects of SO have a number of similarities with those of alcohol. This present study evaluated the pharmacodynamic interaction of SMO.IR (a bioequivalent solid immediate release formulation of SO) and alcohol to test whether this interaction potentiates or diminishes the effects of both SMO.IR and alcohol and to what extent.
Methods: 24 healthy volunteers of either sex participated in a randomized, double-blind, double-dummy, and crossover trial. Study participants randomly received the four study treatments with an at least two-day interval between each administration: a) 2.25 g of SMO.IR and placebo alcohol preparation, b) 2.25 g of SMO.IR and alcohol (0.7 g/kg alcohol for males and 0.57 g/kg alcohol mixed with apple juice), c) 2.25 g of SMO.IR matching placebo and alcohol, d) 2.25 g of SMO.IR matching placebo and placebo alcohol preparation. Study endpoints were objective and subjective cognitive tests, adverse events, vital signs assessed before, and 15 and 165 minutes after oral study drug administration. Cognitive tests included: Body Sway Test, Saccadic Eye Movement, Choice Reaction Time, Critical Tracking Test, Digit Vigilance, Numeric and, Spatial Working Memory, Bond & Lader VAS, ARCI 49, and Biphasic Alcohol Effects Scale.
Results: Alcohol produced the expected significant impairment in cognitive performance as well as the expected subjective sedation rapidly after intake (from 15 min). The effects of a single dose of SMO.IR produced a few significant objective and subjective sedative effects which were more pronounced 165 min post dose. Subjective complaints were related to lesser stimulation and greater sedation, but 165 minutes after administration this subjective sedation feeling was lesser with SMO.IR than with alcohol. There was a significant interaction between SMO.IR and

alcohol, at 15 min, that resulted in an increase in alertness and stimulation and a decrease in sedation. In addition, an isolated mild decrease in digit vigilance accuracy occurred at 165 min post dose after combination.
When SMO.IR was administered together with alcohol there was a slight increase in the total number of the TEAEs (46 in total) than when either SMO.IR alone (30) or alcohol alone were administered (34). No significant changes in heart rate, blood pressure, oxygen saturation, and routine laboratory tests were observed.
Conclusion: SMO.IR and alcohol have a distinct adverse effect profile. Sedative effects of SMO.IR are much less marked than those of alcohol and no reciprocal potentiation was observed.

Disclosures: N. Pross: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biotrial. **N. Fauchoux:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biotrial. **H. Hadjduchova:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; D&A Pharma. **C. Denot:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biotrial. **A. Dufour:** None. **A. Patat:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biotrial. **P. Vivet:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; D&A Pharma.

Poster

544. **Alcohol: Tolerance, Dependence, and Withdrawal**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 544.30/KK4

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant R01AA018799

Title: Glial regulation of alcohol behavioral responses in *Drosophila*

Authors: *S. PARKHURST[1], A. V. LEGENDRE[2], E. C. KONG[3], F. W. WOLF[2,3];
[2]Mol. Cell Biol., [1]Univ. of California, Merced, Merced, CA; [3]Ernest Gallo Clin. and Res. Ctr., Emeryville, CA

Abstract: Increased alcohol intake is facilitated by the development of tolerance, a simple form of behavioral plasticity that can be simply defined as the acquired resistance to the aversive effects of the drug. While the role of neurons in alcohol tolerance is well documented, little is known about the contribution of glia. In the fruit fly Drosophila, glia are thought to perform many of the same roles as their vertebrate counterparts, although much of their function remains unknown. Studies in flies and mice provide evidence that glia and neurons communicate and that glia are crucial for regulation of complex behaviors. We discovered that a glia-specific gene responds to acute ethanol exposure and is required for the development of ethanol tolerance in the glia that make up the blood-brain barrier. These findings led us to test the role of currently known glial functions in ethanol behaviors. Our results indicate that the astrocyte-like glia that are in contact with brain synapses both recieve and transmit signals to promote the development of ethanol tolerance. These data indicate that two distinct types of glia regulate ethanol behaviors and suggest that glial-neuronal communication is crucial for the expression of one form of behavioral plasticity.

Disclosures: S. Parkhurst: None. **F.W. Wolf:** None. **A.V. Legendre:** None. **E.C. Kong:** None.**Poster**

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.01/KK5

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH grant R43 DA031578,

NIH grant R44 DA031578.

NIH grant 2R44DA026363

Title: Nicotine aerosol delivery to rodents in a self-administration model mimics nicotine intake during cigarette smoking and can be used to screen smoking cessation pharmacotherapies

Authors: X. M. XIE[1], L. YANG[1], C. PASCUAL[1], B. ZOU[1], A. MALIK[1], L. TOLL[2], N. ZAVERI[3], Y. ZHU[4], *X. M. SHAO[5];

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Abstract: Cigarette smoke is an aerosol with tiny particles that contain nicotine. Smoke aerosol particles within the respirable size range carry nicotine into the alveoli of the lungs, leading to a rapid increase in arterial blood nicotine concentrations and nicotine reaches the brain in 8-10 s. The high-arterial-peak concentration pattern of nicotine pharmacokinetics plays an important role in development of dependence. The determination of efficacy for smoking cessation drugs under development typically uses the intravenous (i.v.) self-administration (SA) of nicotine in freely-moving rats. The i.v. SA is invasive and does not recapitulate the nicotine delivery route of cigarette smoking. To generate higher predictive validity in nicotine addiction models, we developed a novel system for delivering nicotine to rodents using alveolar region-targeted aerosol technology. The aerosol SA chamber consists of the SmartCageTM, a versatile homecage behavior monitoring system, equipped with special software which allows nicotine aerosol delivery to rodents trained to self-administer nicotine by pressing levers or by a scheduled passive delivery. The system also consists of a nebulizer, a food dispenser, two flowmeters and a pressure gauge. The aerosol droplet size distribution was determined that had a mass median aerodynamic diameter of 2.03 μm with a geometric standard deviation of 1.52, which is within the range of respirable diameter. With aerosol generated using 0.5 % nicotine solution in the nebulizer, the time-course of arterial plasma nicotine concentrations during and after 2 min aerosol inhalation in rats showed a peak level of 57 ng/ml within 1 to 3 min from the start of aerosol exposure and the nicotine level declined over the next 20 min. The magnitude and kinetics mimic humans smoking a cigarette. By operating levers rats obtain nicotine aerosol and develop dependency over a period of 3-4 weeks. This system was evaluated by investigating whether the clinically used smoking cessation pharmacotherapy, varenicline, and a recently published alpha3beta4 nicotinic acetylcholine receptor (nAChR) ligand, AT-1001, which have both been shown to inhibit nicotine self-administration in a typical i.v. SA paradigm, also showed efficacy when nicotine was delivered via aerosol in our SA chamber. Both varenicline and AT1001 reversibly blocked nicotine aerosol SA similar to their action using i.v. nicotine SA. Enhancement of the system and SA validation studies are currently ongoing in order to develop predictive in vivo screening systems for phenotypic drug discovery for smoking cessation.

Disclosures: X.M. Xie: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AfaSci, Inc. Redwood City, CA,. **L. Yang:** None. **C. Pascual:** None. **B. Zou:** None. **A. Malik:** None. **L. Toll:** None. **N. Zaveri:** None. **Y. Zhu:** None. **X.M. Shao:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.02/KK6

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA020686 to PJK

NIH Grant DA032543 to CDF

Title: Hypocretin modulation of nicotine-mediated alterations in brain reward function are dependent on required level of effort

Authors: *C. D. FOWLER, P. J. KENNY;
Mol. Therapeut., The Scripps Res. Inst., JUPITER, FL

Abstract: Our laboratory has previously shown that hypocretin (orexin) modulates nicotine reinforcement in rodents. Specifically, blockade of hypocretin receptors in rats or genetic ablation of the hypocretin-1 receptor gene in mice results in decreased motivation to self-administer nicotine. Since other reports with varying experimental conditions have not demonstrated alterations in behavioral responding for nicotine with blockade of hypocretin-1 receptors, we sought to more fully investigate the involvement of hypocretin on brain reward function under varying effort requirements. As such, we examined the effects of the selective hypocretin-1 receptor antagonist SB-334867 on intracranial self-stimulation (ICSS) thresholds and intravenous nicotine self-administration (SA) in rats while manipulating effort requirements for each procedure. Adult male Wistar rats were surgically implanted with bipolar cranial electrodes and subsequently trained in a discrete trial current threshold ICSS procedure. Concurrently, subjects were also trained to press an active lever to receive food reward under a fixed ratio 5, time out 20 sec schedule of reinforcement (FR5 TO20sec). Thereafter, the rats were surgically implanted with intravenous catheters into the right jugular vein. After a recovery period, the subjects were permitted daily access to respond for intracranial self-stimulation (pre-SA ICSS), followed by an 1-hr nicotine SA session, and subsequently a second self-stimulation session (post-SA ICSS). After achieving stable levels of responding, the procedural requirements were manipulated to make the task less rigorous. For nicotine SA, the FR5 requirement was modified to a FR1 requirement; for ICSS, the number of trials per current intensity, inter-trial interval duration, and within-trial duration were decreased. We found that administration of SB-334867 decreased nicotine self-administration under the more effortful FR5 SA conditions, as previously reported, but was ineffective under the less strenuous FR1 requirements. Further, under the more effortful ICSS conditions, SB-334867 significantly attenuated the nicotine-induced lowering of brain reward thresholds, whereas when the procedure conditions required less rigorous, SB-334867 was ineffective in altering nicotine-induced lowering of brain reward

thresholds. Together, these data indicate that hypocretin signaling mediates nicotine's effects on brain reward function only when higher levels of motivation are required, and as such, therapeutics targeting hypocretin transmission for smoking cessation may be more efficacious when access to the drug is limited.

Disclosures: C.D. Fowler: None. **P.J. Kenny:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.03/KK7

Topic: C.18. Drugs of Abuse and Addiction

Support: DA024932 and DA031222 to J.A.H

DA020686 and DA023915 to PJK

Title: A hypocretin-regulated "value attribution" circuit in dorsal thalamus controls compulsive nicotine use

Authors: *J. A. HOLLANDER, B. R. LEE, L. M. TUESTA, E. KOESEMA, P. J. KENNY; The Scripps Res. Inst., JUPITER, FL

Abstract: Tobacco addiction occurs when the motivation to obtain nicotine reward persists at the expense of alternative sources of reward, but the underlying mechanisms remain poorly understood. Hypocretin (Hcrt) is a recently discovered lateral hypothalamic neuropeptide that is emerging as an important regulator of reward and motivation. While Hcrt has been hypothesized to control nicotine by modulating its rewarding properties, we investigated the possibility that Hcrt instead regulates the motivational "value" of nicotine, reflected in the amount of effort that will be expended (price paid) to obtain the drug. First, we found that that mice with a null mutation in the Hcrt-1 receptor decreased intravenous nicotine self-administration (SA) compared to their wildtype littermates (WT) when the fixed-ratio schedule was increased (which requires greater effort to receive drug reward). Using a Hcrt-1 receptor reporter mouse line (OX1-eGFP) we identified a previously uncharacterized population of GABAergic cells in the thalamus located adjacent to the lateral habenula. Intriguingly, we found that virus-mediated re-expression of these genetically ablated receptors in this region entirely rescued the deficits in nicotine intake observed in the KO mice. Further, thalamic infusion of the Hcrt-1 receptor antagonist, SB-334867 decreased nicotine SA. Based on these observations, we hypothesized that disruption of Hcrt-1 transmission may result in local thalamic hyperexcitability, which

decreases the value of nicotine without impacting its rewarding effects. Consistent with this idea, increased excitability of local thalamic activity by Designer Receptors Exclusively Activated by Designer Drugs (DREADD) reduced nicotine SA, whereas DREADD-mediated inhibition of activity triggered compulsive-like responding even when its delivery was accompanied by aversive footshock. Our findings identify a novel hypocretin-controlled thalamic circuit that attributes value to nicotine by allocating resources to obtain the drug. Further, excessive activation of this pathway may explain why smokers persist in the habit despite awareness of the potential fatal consequences.

Disclosures: J.A. Hollander: None. **B.R. Lee:** None. **L.M. Tuesta:** None. **E. Koesema:** None. **P.J. Kenny:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.04/KK8

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH DA020686 to PJK

NIH DA032225 to LMT

Title: Brain glucagon-like peptide-1 regulates the reinforcing properties of nicotine

Authors: *L. M. TUESTA, C. D. FOWLER, B. R. LEE, P. BALI, Q. LU, P. J. KENNY; Mol. Therapeut., The Scripps Res. Inst., Jupiter, FL

Abstract: Cigarette smoking is a principal cause of preventable death and disease in developed nations, with approximately $160 billion being spent yearly in the United States to cover direct health care costs from resulting diseases. Nicotine is the major reinforcing component in tobacco smoke that contributes to the development of the harmful smoking habit in humans. The reinforcing actions of nicotine are mediated through various subtypes of nicotinic acetylcholine receptors located within brain reward circuitries. Central glucagon-like peptide-1 (GLP-1) is a neuropeptide produced in the nucleus of the solitary tract (NTS), a brain stem structure abundant in nicotinic acetylcholine receptors. Whilst noradrenergic efferents from the NTS have been implicated in drug dependence processes, the potential contribution of GLP-1 output from the NTS in this process has not been investigated.
We found that GLP-1 receptor KO mice consume more nicotine when compared to wildtype

littermates, across all doses tested. Conversely, GLP-1 neuron-specific stimulation via cre-inducible M3 DREADDs show that brain-derived GLP-1 reduces the reinforcing properties of nicotine. Our results suggest that GLP-1 transmission serves as a negative regulator of nicotine reinforcement. Recently, our laboratory reported that excitatory inputs from the medial habenula to the interpeduncular nucleus (IPN) also serve to negatively regulate nicotine intake. Intriguingly, GLP-1 receptors are densely expressed in IPN. Electrophysiological recordings coupled with optogenetic or electrically evoked activity revealed that GLP-1 receptors located on presynaptic terminals enhanced activity of IPN neurons. Further, stimulation of GLP-1 receptors in IPN via microinfusion of the GLP-1 receptor agonist, Exendin-4, reduces nicotine intake in rats through a cAMP-dependent mechanism.
These data identify a new brain circuit - IPN-projecting GLP-1 neurons - that negatively regulate nicotine intake. Moreover, these findings suggest that modulation of GLP-1 receptor transmission may be viable target for the development of novel therapeutics for smoking cessation.

Disclosures: L.M. Tuesta: None. **C.D. Fowler:** None. **B.R. Lee:** None. **P. Bali:** None. **Q. Lu:** None. **P.J. Kenny:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.05/LL1

Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA DA–03977

Title: Inactivation of the central nucleus of the amygdala reduces the stress-induced amplification of relapse to nicotine-taking

Authors: *G. YU, Z. HUANG, H. CHEN, B. M. SHARP;
Pharmacol., Univ. of Tennessee Hlth. Sci. Ctr., MEMPHIS, TN

Abstract: Quitting smoking is very challenging and often unsuccessful due to multiple factors that promote relapse after abstinence. Stress is an important factor that increases the likelihood of relapse. In rodents, stress-induced reinstatement of nicotine-seeking (non-reinforced) after extinction of operant nicotine self-administration (SA) is well established. Recently, we established an animal model demonstrating the amplification of relapse to nicotine-taking (reinforced) by repetitive exposure to stress during abstinence. However, the underlying mechanism is unknown. The central nucleus of the amygdala (CNA) plays a critical role in

stress-induced reinstatement of nicotine-seeking. Therefore, we determined the effect of CNA inactivation on the amplification of relapse to nicotine-taking by stress. Adult male SD rats acquired nicotine SA (0.03 mg/kg, 23h/d) under an FR 5 schedule of reinforcement. Restraint stress (30 min) was administered on alternate days (total of 4 stress sessions) during abstinence from nicotine SA. Thereafter, animals reacquired nicotine SA, beginning 24 h after the final stress. A mixture of baclofen and muscimol (B/M = GABAb and GABAa agonists; 0.3 and 0.03 nmol/side) was injected into CNA 30 min prior to the nicotine SA test session (i.e.,reacquisition). Withdrawal per se increased the reacquisition of nicotine SA (i.e., active lever presses and nicotine intake; $p<0.05$). Repetitive stress further amplified the reacquisition of nicotine SA ($p<0.05$), and induced nicotine-taking during the behaviorally inactive phase (i.e., lights on) of the 24 h light/dark cycle. In rats exposed to repetitive stress, inactivation of CNA reduced active lever presses and nicotine intake ($p<0.05$) to their pre-withdrawal baseline levels. These data show that CNA is a critical neural substrate involved in the amplification of relapse to nicotine-taking by chronic stress.

Disclosures: G. Yu: None. **Z. Huang:** None. **H. Chen:** None. **B.M. Sharp:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.06/LL2

Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA DA–03977

Title: Histone deacetylase inhibition amplifies reacquisition of nicotine SA and contributes to the stress-induced amplification of relapse to nicotine-taking

Authors: G. YU, Z. HUANG, H. CHEN, *B. M. SHARP;
Dept Phamacol, Univ. Tennessee Hlth. Sci. Ctr., MEMPHIS, TN

Abstract: Stress promotes relapse to smoking. Recently, we established an animal model demonstrating the amplification of relapse to nicotine-taking by stress during abstinence from nicotine SA. Further studies of this model may elucidate mechanisms underlying stress-induced relapse to smoking. Epigenetic modifications, such as histone acetylation, are involved in the long-term effects of both drugs of abuse (i.e. cocaine) and stress. However, the potential role of epigenetic mechanisms in the stress-induced amplification of relapse to nicotine-taking is unknown. Therefore, we determined the effect of histone deacetylase (HDAC) inhibitors on the reacquisition of nicotine SA in rats exposed to repetitive stress during abstinence. Adult male SD

rats acquired nicotine SA (0.03 mg/kg, 23h/d) under an FR 5 schedule of reinforcement. Restraint stress (30 min) was administered on alternate days (total of 4 stress sessions) during abstinence from nicotine SA. Thereafter, animals reacquired nicotine SA, beginning 24 h after the final stress. HDAC inhibitors, 4-phenylbutyrate (4-PB, 20 mg/kg) or trichostain A (TSA, 0.3 mg/kg) were administered (i.v.) 30 min prior to each restraint or sham-stress session. Neither 4-PB or TSA altered the effects of stress on reacquisition of nicotine SA (i.e., active lever presses and nicotine intake; $p>0.05$). In contrast, an injection of 4-PB or TSA on alternate days of abstinence (total of 4 injections) amplified the reacquisition of nicotine SA in unstressed rats ($p<0.05$). These data show that inhibition of histone deacetylase amplifies relapse to nicotine-taking to the same extent as stress, but does not alter the enhancing effects of stress. Therefore, the effect of stress on relapse to nicotine-taking may be mediated by inhibition of histone deacetylase.

Disclosures: G. Yu: None. **B.M. Sharp:** None. **Z. Huang:** None. **H. Chen:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.07/LL3

Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA Grant# 1F31DA034449-01A1

Title: Interoceptive conditioning with the nicotine stimulus slowed by lesions to posterior but not anterior dorsomedial striatum

Authors: *S. CHARNTIKOV, S. PITTENGER, K. VESTAKIS, R. A. BEVINS;
Dept of Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Tobacco use is the leading cause of preventable deaths worldwide. This habit is not only debilitating to individual users but also to those around them (second-hand smoking). Nicotine is the main addictive component of tobacco products and is a potent stimulant and a reinforcer. Importantly, besides its unconditional effects, nicotine also has conditional stimulus effects that may contribute to the tenacity of the smoking habit. Investigation of learning processes involving nicotine as a conditioned stimulus (CS) is an understudied area relevant to nicotine dependence. A preliminary study assessing neurobiological loci involved in nicotine-evoked conditioned response (CR) found that activation (as revealed through elevation of c-Fos) of dorsomedial caudate putamen (dmCPu) was dependent on learning history with nicotine. Therefore, the current study examined the effect of permanent neurotoxin lesions of either

anterior (a-) or posterior (p-) dmCPu on acquisition of the nicotine-evoked CR. In this experiment, rats received excitotoxic (NMDA) or sham lesions (vehicle) of either a-dmCPu or p-dmCPu before nicotine CS training. During nicotine CS training (20 total daily sessions), the nicotine stimulus (0.4 mg/kg; injected SC) was paired 100% of a time with intermittent access to sucrose deliveries (36 per session); sucrose was not available on intermixed saline days. Using this protocol, the nicotine CS readily acquired control of a goal-tracking CR (anticipatory food-seeking response) in the shams and in the a-dmCPu lesioned animals. Acquisition of conditioned responding was slowed in rats with lesions to p-dmCPu. Thus, permanent excitotoxic lesions to p-dmCPu and not a-dmCPu blunted acquisition of conditioned association between the nicotine stimulus and the appetitive sucrose reward. This finding, in part, supports an assumption of compartmentalized involvement of dmCPu in the acquisition and expression of conditioned behaviors where the posterior portion of dmCPu is predominantly involved in the acquisition and the anterior portion in expression. A follow-up experiment is underway in our laboratory to determine whether the a-dmCPu is involved in the expression of appetitive interoceptive conditioning with the nicotine stimulus.

Disclosures: S. Charntikov: None. **S. Pittenger:** None. **K. Vestakis:** None. **R.A. Bevins:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.08/LL4

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA034389

Title: The effect of switching pharmacological intervention on nicotine-evoked conditioned responding in extinction

Authors: *S. T. PITTENGER, L. C. ZEPLIN, R. A. BEVINS;
Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Nicotine dependence is a costly economic and societal burden. Several pharmacotherapies have been developed to aid in smoking cessation. While these medications increase successful quit attempts compared to no cessation aid, attempts to quit often fail. A relapse or threat of relapse to smoking is often met with a change in the smoking cessation aid. Although this practice is becoming common, very little is known about the behavioral or pharmacological effects of switching pharmacotherapies. To begin to fill this gap, the present

study examined the effects of switching pharmacotherapies on interoceptive conditioning involving the nicotine stimulus. This question is of import given the role interoceptive stimuli play in addiction maintenance and relapse.
Utilizing a discriminated goal-tracking task, male Sprague Dawley rats (n=60) were trained to discriminate nicotine from saline. We paired 0.4 mg/kg nicotine injections (SC) with intermittent sucrose access during a 20-min session in a conditioning chamber. On intermixed days, saline (0.9%; SC) was administered before sessions and no sucrose available. Following discrimination training was the extinction phase. Rats receive their assigned ligand before the start of the 20-min session and sucrose was no longer available. Rats were separated into 5 groups for this extinction phase: Sal-to-Sal, Nic-to-Nic, 5.6Nor-to-5.6Nor, 5.6Nor-to-1.0Var, and 5.6Nor-to-0.3Var (n=12 per group; Sal = saline; Nic = 0.4 mg/kg nicotine; 5.6Nor = 5.6 mg/kg nornicotine; 1.0Var = 1.0 mg/kg varenicline; 0.3Var = 0.3 mg/kg varenicline). The first part of group names denote what drug was administered on the first 3 days of extinction training; the second part represent what was administered on the last 3 days of extinction. We also investigated how this extinction history with stimuli that share stimulus effects with nicotine would affect appetitively-motivated behavior controlled by nicotine establishing a cumulative dose-effect curve.
Rats readily discriminated nicotine from saline sessions with nicotine evoking a robust goal-tracking response. Nornicotine partially substituted for nicotine in the early extinction sessions. Rats that were switched from nornicotine to varenicline displayed attenuated responding relative to those that continued to receive nornicotine throughout extinction. This suggests that early extinction with nornicotine is sufficient to extinguish nicotine-like responding evoked by varenicline. These group differences in extinction, however, did not translate into difference in the cumulative nicotine dose-effect curve on the transfer test.

Disclosures: S.T. Pittenger: None. **L.C. Zeplin:** None. **R.A. Bevins:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.09/LL5

Topic: C.18. Drugs of Abuse and Addiction

Support: UTHSC faculty startup fund

Title: Cooling sensation of menthol is a conditioned reinforcer for nicotine

Authors: *H. CHEN, T. WANG, B. WANG;
Dept Pharmacol, Univ. Tennessee Hlth. Sci. Ctr., MEMPHIS, TN

Abstract: Menthol is the most common tobacco additive. It induces multimodal sensory stimulation that includes a cooling sensation, minty odor, and bitter taste. Approximately 30% US smokers prefer menthol cigarettes over non-menthol ones. The use of menthol in cigarettes facilitates smoking initiation and enhances nicotine dependence. Smoking menthol cigarettes is also associated with less responsiveness to medication and greater difficulty in quitting, especially in minority ethnic populations. Despite the rapid accumulation of clinical data, a mechanistic understanding of menthol's effect is still lacking, partly due to the lack of animal models. We hypothesized that the cooling sensation of menthol, but not its olfactogustatory stimulation, is a conditioned reinforcer for nicotine reward. We tested the hypothesis by using licking as the operant behavior for nicotine self-administration (SA). Adolescent female Sprague-Dawley rats were given daily 3 h SA sessions with two drinking spouts; licking on the active spout delivered 60 µl menthol (0.01% w/v) and i.v. nicotine (30 µg/kg/infusion) on a fixed-ratio 10 reinforcement schedule. Licking on the inactive spout had no programmed consequence. The number of infusions significantly increased ($p < 0.005$) over the test sessions, gradually reached ~10 infusions around day 7, and maintained that level thereafter. In contrast, rats self-administered saline with menthol cue or nicotine with vehicle as cue (0.01% Tween 80) failed to shown an increase in nicotine infusion (ps < 0.05). The number of infusions was significantly higher in nicotine menthol rats compared to saline menthol ($p<0.001$) and nicotine-vehicle ($p<0.001$) rats, demonstrating that menthol as a sensory cue supports nicotine SA. Rats yoked to the nicotine-menthol group emitted significantly fewer active licks ($p<0.01$). Unsweetened grape Kool-Aid (0.1% w/v), a flavor that is mostly odor did not support nicotine SA. These rats avoided the active spout ($p < 0.001$) and self-administered less than 2 infusions per session. Last, WS-23, a compound induces similar cooling effect as menthol but has no detectable odor also supported nicotine SA (active lick > inactive lick, $p < 0.05$, mean infusion = 11.4 ± 1.3 for the last four days). Together, these data demonstrated that although menthol per se is not reinforcing, classical conditioning between its cooling, but not the olfactogustatory properties, with nicotine enhances the motivation to obtain the drug.

Disclosures: H. Chen: None. **T. Wang:** None. **B. Wang:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.10/LL6

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA026894

Title: Carbon Disulfide mediates socially-acquired nicotine self-administration

Authors: *S. GONG, T. WANG, H. CHEN;
Dept pharmacology, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Social environment plays critical roles in smoking initiation. The majority of first time cigarette uses occurred in the presence of friends; peer smoking was one of the strongest predictors of smoking initiation. Further, befriend with smokers increased the likelihood of becoming a smoker, while discouraging from the social network was negatively associated with smoking status. We previously reported that rats developed conditioned taste aversion (CTA) to an appetitive olfactogustatory (OG) cues contingent with self-administered i.v. nicotine. However, sustained self-administration (SA) of nicotine was developed in the presence of a demonstrator rat that had access to the same OG cue (but not receiving nicotine). This socially-acquired SA behavior required nicotine contingent odor cue but the signal mediating social learning was unknown. We hypothesized that carbon disulfide, a chemical detected in rats' breath and involved in social transmission of food preference, also mediates socially-acquired nicotine SA. We tested this hypothesis by placing solo rats in operant chambers equipped with two lickometers. Licking on the active spout meeting fixed-ratio 10 schedule resulted in the simultaneous delivery of an olfactogustatory cue (0.4% saccharin and 0.1% unsweetened grape Kool-Aid) containing 0.01% carbon disulfide to the active spout and i.v. nicotine (30 µg/kg/inf). In contrast to the development of CTA, these rats self-administered an average of 10.3 ± 1.0 nicotine infusions per 3 h daily sessions during the last three days of test, which was almost identical to the number of infusions self-administered by rats placed with demonstrator rats. Repeated measures ANOVA found a significant interaction between session number and spout ($p<0.001$) in rats had access to the OG cue and carbon disulfide. During sessions 1-5, the number of active licks was significantly less than inactive licks ($p<0.05$), while during sessions 6-10, the number of active licks were higher than inactive licks ($p=0.05$). Therefore, these data demonstrated for the first time that carbon disulfide mediates socially-acquired nicotine SA.

Disclosures: S. Gong: None. **T. Wang:** None. **H. Chen:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.11/LL7

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant K01 DA030445

Pilot Grant P50-CA-143187 from the Center for Interdisciplinary Research on Nicotine Addiction at UPenn

Institutional Research Grant IRG-78-002-31 from the American Cancer Society and the Abramson Cancer Center at UPenn

Title: Acute administration of the α4β2* nAChR agonist ABT-089 and the α7 nAChR agonist ABT-107 attenuates nicotine seeking in rats

Authors: *B. A. KIMMEY, A. C. ARREOLA, A. M. LEE, H. D. SCHMIDT;
Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Nicotine addiction is a chronic relapsing disorder that represents the single-most preventable cause of death in the United States today. While two-thirds of the tobacco-smoking population expresses a desire to quit, only 3% of smokers successfully quit on their own. Current FDA-approved smoking cessation medications maintain long-term smoking abstinence in only 25% of smokers attempting to quit. Therefore, there is a critical need to develop more efficacious smoking cessation medications. Nicotine is the primary psychoactive component in tobacco smoke that binds to and stimulates nicotinic acetylcholine receptors (nAChRs). A large body of evidence indicates that increased nAChR signaling plays a critical role in nicotine self-administration and the reinstatement of nicotine-seeking behavior in rats, an animal model of nicotine craving and relapse in human smokers. Here, we examined the effects of acute administration of the α4β2* nAChR agonist ABT-089 and the α7 nAChR agonist ABT-107 on nicotine taking and seeking in rats. Rats were trained to self-administer intravenous infusions of nicotine (30 μg/kg/59 μL). Acute administration of ABT-089 (0, 1.2 and 12.0 mg/kg, i.p.) and ABT-107 (0, 0.03 and 0.3 mg/kg, i.p.) prior to the start of an operant session had no effect on self-administration of nicotine when rats were maintained on a fixed-ratio 5 (FR5) schedule of reinforcement. In contrast, acute administration of ABT-089 (12 mg/kg, i.p.) or ABT-107 (0.3 mg/kg, i.p.) prior to a nicotine reinstatement test session attenuated nicotine-seeking behavior. These effects did not generalize to other reinforced behaviors, as neither ABT-089 nor ABT-107 affected sucrose self-administration or the reinstatement of sucrose seeking. This is the first study to assess the effects of ABT-089 and ABT-107 on nicotine self-administration and reinstatement of nicotine seeking in rats. Our results indicate that stimulation of nAChRs during withdrawal is sufficient to attenuate nicotine seeking.
This work is supported by K01 DA030445 (H.D.S.), a pilot grant (P50-CA-143187) from the Center for Interdisciplinary Research on Nicotine Addiction (CIRNA) at UPenn (H.D.S.), and an Institutional Research Grant (IRG-78-002-31) from the American Cancer Society and the Abramson Cancer Center at UPenn (H.D.S.).

Disclosures: B.A. Kimmey: None. **A.C. Arreola:** None. **A.M. Lee:** None. **H.D. Schmidt:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.12/LL8

Topic: C.18. Drugs of Abuse and Addiction

Support: K01 DA030445

P50-CA-143187

IRG-78-002-31

Title: Repeated administration of an acetylcholinesterase inhibitor attenuates nicotine taking in rats

Authors: *A. C. ARREOLA, B. A. KIMMEY, L. E. RUPPRECHT, A. M. LEE, M. R. HAYES, H. D. SCHMIDT;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Nicotine craving and cognitive impairments represent core symptoms of nicotine withdrawal that predict relapse in abstinent smokers. These findings suggest that cognitive-enhancing medications may prevent drug craving and relapse, in part, by reversing or normalizing nicotine withdrawal-induced cognitive impairments. Acetylcholinesterase inhibitors (AChEIs) are FDA-approved to treat cognitive deficits in patients with Alzheimer's disease. Previously, we demonstrated that acute administration of the AChEIs galantamine and donepezil attenuates nicotine taking and seeking in rats. However, chronic studies of AChEI administration on nicotine taking are needed in order to model the dosing regimen that is likely to be prescribed to smokers attempting to quit. Therefore, the goal of these experiments was to determine the effects of repeated AChEI administration on nicotine self-administration in rats, an animal model of voluntary nicotine taking in human smokers. Rats were trained to self-administer intravenous infusions of nicotine (0.03 mg/kg/0.59mL) on a fixed-ratio 5 (FR5) schedule of reinforcement. Once rats maintained stable nicotine taking, galantamine (0, 0.5 and 5.0 mg/kg, i.p.) was administered 20 minutes prior to 10 consecutive daily nicotine self-administration sessions. Repeated administration of 5.0 mg/kg galantamine attenuated nicotine taking in rats. In order to determine if the effects of repeated galantamine generalize to other reinforced behaviors, a separate group of rats was trained to self-administer sucrose pellets. No differences in sucrose self-administration were noted between rats pretreated with galantamine when compared to saline treated controls. Malaise symptoms, including nausea and vomiting, are commonly reported adverse effects of AChEIs administration in humans. Therefore, the effects of repeated galantamine administration on ad libitum food intake and pica, an animal model that is used to

assess rodent consumption of non-nutritive substances (i.e. kaolin clay) in response to emetic agents, were tested in a separate cohort of rats. No effects of repeated galantamine administration on pica or normal feeding behavior were observed. Taken together, these results indicate that repeated AChEI administration attenuates nicotine taking and that these effects are not due to adverse malaise-like symptoms.
This work is support by K01 DA030445 (H.D.S.), a pilot grant (P50-CA-143187) from the Center for Interdisciplinary Research on Nicotine Addiction (CIRNA) at UPENN and an Institutional Research Grant (IRG-78-002-31) from the American Cancer Society and the Abramson Cancer Center at UPENN (H.D.S.)

Disclosures: A.C. Arreola: None. **B.A. Kimmey:** None. **L.E. Rupprecht:** None. **A.M. Lee:** None. **M.R. Hayes:** None. **H.D. Schmidt:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.13/LL9

Topic: C.18. Drugs of Abuse and Addiction

Title: Juvenile play experience attenuates the initial level of nicotine consumption

Authors: *B. T. HIMMLER[1], E. SNOW[2], A. MCMICKLE[2], S. M. PELLIS[1], B. KOLB[1], K. D. BIONDOLILLO[2];
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Abstract: Nicotine given in adulthood increases the dendritic arbor of the neurons of the medial prefrontal cortex (mPFC). Juvenile play experience increases this neural response to nicotine and behavioral testing for drug sensitization suggests that this play-induced plasticity initially attenuates the effects of nicotine. The objective of the present study was to determine if prior play experience influences the voluntary consumption of nicotine. Post-weaning rats were reared with either three other juvenile rats (play group) or one adult female rat (no play group), and in adulthood, half of each group was exposed to nicotine. Following this exposure, all groups received a multiple bottle arrangement consisting of two concentrations of nicotine (5µg/ml or 8µg/ml) and water, which were all freely available for consumption. The total amount consumed of each was measured. While all groups preferred the lower dose (5µg/ml) compared to the higher dose (8µg/ml) of nicotine, animals with prior play experience exhibited an initial attenuation in their consumption of nicotine. Therefore, the play-induced plasticity of the mPFC

appears to serve, at least initially, as a protective change both in the sensitization to nicotine and also in the consumption of nicotine.

Disclosures: B.T. Himmler: None. **E. Snow:** None. **A. McMickle:** None. **S.M. Pellis:** None. **B. Kolb:** None. **K.D. Biondolillo:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.14/LL10

Topic: C.18. Drugs of Abuse and Addiction

Support: Virginia Families for Healthy Youth

Virginia Tobacco Settlement Foundation

Virginia Youth Tobacco Programs/VCU

Title: Loss of Cd81 function increases nicotine preference, but decreases depression- and anxiety-like behavior in mice

Authors: R. L. MURPHY, L. L. LOCKLEAR, M. H. NIAZ, R. WALTON, *K. J. FRYXELL; Sch. of Systems Biol., George Mason Univ., MANASSAS, VA

Abstract: There are poorly understood connections between mental health disorders and drug abuse, likely reflecting both causes (i.e., some mental health disorders cause increased emotional needs and/or vulnerability to drug dependence) and effects (i.e., drug addiction may produce negative emotional or cognitive effects). As an example, nicotine dependence has been shown to be statistically associated with depression, anxiety, schizophrenia, and ADHD. Our laboratory and others have previously shown that the *Cd81* gene is implicated in the behavioral and gene expression response to drugs of abuse, including cocaine and nicotine, but does not affect cognitive abilities in the Morris water maze. Here we show that the loss of *Cd81* function significantly increased the voluntary nicotine consumption of adult female C57BL/6J mice. The voluntary nicotine consumption of both *Cd81* -/- and +/+ mice increased steadily for 4 weeks, and then reached a plateau that was maintained for another 4 weeks, suggesting that *Cd81* did not affect the aversion to higher doses of nicotine. We further show that loss of *Cd81* function significantly increased struggling in the forced swim test and the tail suspension test, two models of depression. Finally, loss of *Cd81* function in male mice significantly increased their preference for the light side of the light/dark box, a model of anxiety. These results indicate that

CD81 (an adaptor protein involved in assembly of signaling complexes) plays a key role in both drug reward and emotional behaviors.

Disclosures: R.L. Murphy: None. **K.J. Fryxell:** None. **L.L. Locklear:** None. **M.H. Niaz:** None. **R. Walton:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.15/LL11

Topic: C.18. Drugs of Abuse and Addiction

Support: CIHR

Title: Nicotine enhances conditioned approach behavior measured by a pavlovian autoshaping procedure and responding for conditioned reinforcement

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[1]Psychology, Univ. of Toronto, Toronto, ON, Canada; [2]Psychiatry, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Nicotine reinforcement is thought to be, at least in part, due to an effect on enhancing the attractive and motivating properties of other, non-nicotinic reward stimuli. Our lab and others have shown that nicotine facilitates approach to a reward delivery receptacle in the presence of a conditioned stimulus (CS) during Pavlovian association trials, but this latter effect is not always observed. This may be due to differences in conditioned response trajectories under the influence of nicotine, with some animals attracted toward the CS itself rather than the goal receptacle. 40 water-restricted rats received nicotine (n = 20) or saline (n = 20) injections prior to 6 daily Pavlovian autoshaping trials, where an illuminated lever-CS was inserted into the test chamber just prior to the delivery of a water reinforcer. Animals that received nicotine exhibited higher levels of conditioned responding directed toward the lever-CS. Next, a subgroup of nicotine-exposed (n = 10) and saline-exposed (n = 10) animals were switched to saline or nicotine pretreatments for 6 additional trials. Removing nicotine decreased responding on the lever. Responding in the water receptacle was unaffected by nicotine. In a test of the motivating properties of the lever-CS, presentations of the lever supported the acquisition of a new operant response and nicotine enhanced this effect in animals with a history of nicotine exposure. Together, these data suggest that nicotine reinforcement is dually influenced by effects on enhancing both the attractive and motivating properties of CSs.

Disclosures: E.G. Guy: None. **P.J. Fletcher:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.16/LL12

Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA- P01 DA017259

Research Supplement to Promote Diversity in Health-Related Research- RO1 AA020404

Title: Enhanced nicotine reward in a mouse model of the P129T FAAH gene polymorphism

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[1]Committee on the Neurobio. of Addictive Disorders, [2]Dept. of Chem. Physiol., The Scripps Res. Inst., La Jolla, CA

Abstract: A single nucleotide polymorphism (SNP) of the human fatty acid amide hydrolase (FAAH) gene leads to a missense mutation that substitutes a proline residue for threonine (P129T; C385A). The product of this mutation is a variant of the FAAH protein, an enzyme involved in the metabolism of anandamide and other fatty acid ethanolamides. Clinical studies report an association between the P129T mutation and problem drug use, including nicotine. The current study employed a P129T knock-in (KI) mouse model to characterize the effects of this SNP on the rewarding effects of nicotine using conditioned place preference (CPP), intravenous self-administration (IVSA), and in-vivo microdialysis procedures. For the CPP study, P129T KI and wild-type (WT) mice received 3 days of drug conditioning with both nicotine (0 - 0.7 mg/kg, sc) and saline injections paired with distinct environments in a 3-chamber maze each day. On the test day, the mice were allowed to explore all chambers of the maze in a drug-free state, and the time spent in the drug-paired environment was indexed as a measure of drug reward. In the IVSA studies, the nicotine dose-effect function (0.05 - 0.25 mg/kg/infusion) was characterized under an FR-1 schedule in P129T KI and WT mice, followed by evaluations of the motivation for nicotine intake under a progressive ratio (PR) schedule of reinforcement. The influence of CB1 receptor signaling in nicotine reward was investigated in pretreatment tests employing the CB1 antagonist/ inverse agonist SR141716A (0.1 - 3 mg/kg). The microdialysis study employed a nicotine exposure regimen identical to the conditioning procedure in the CPP study, with nucleus accumbens (NAc) microdialysates collected on the third and final day of drug exposure, and subsequently assayed for monoamine content. The results revealed genotypic differences

such that P129T KI mice exhibit enhanced sensitivity to low doses of nicotine reward relative to WT in the CPP paradigm. P129T KI mice also acquired nicotine IVSA more quickly, displayed an upward shift in the dose response, and achieved higher breakpoints during the PR probe. Pretreatment with SR141716A dose-dependently reduced nicotine IVSA in both mouse strains, but did not alter the genotypic difference in nicotine intake. Finally, P129T mice exhibit enhanced nicotine-induced elevations in NAc DA levels than their WT counterparts. Collectively, these data suggest that nicotine reward is enhanced in mice expressing the P129T mutation. The mechanisms by which genetic disruption of FAAH enhance sensitivity to nicotine do not appear to involve CB1 receptors, but likely involve dopamine transmission in terminal regions of the mesolimbic pathway.

Disclosures: L.A. Natividad: None. **I.Y. Polis:** None. **D.G. Stouffer:** None. **B.F. Cravatt:** None. **L.H. Parsons:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.17/MM1

Topic: C.18. Drugs of Abuse and Addiction

Support: State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development

Title: Neuropathic pain alters nicotine self-administration in rats

Authors: A. CIPPITELLI, J. SCHOCH, D. MERCATELLI, J. WU, K. GAIOLINI, *L. TOLL; Torrey Pines Inst. For Mol. Studies, Port Saint Lucie, FL

Abstract: Tobacco addiction and chronic pain represent highly prevalent and comorbid conditions that engender considerable burdens upon individuals and systems. Experimental studies suggest that nicotine has analgesic properties while epidemiologic evidence shows that smoking is a risk factor for chronic pain. Here, we tested the hypothesis that chronic/neuropathic pain alters the abuse potential of nicotine. Sprague Dawley rats were implanted with intravenous catheters and subjected to L-5 spinal nerve ligation (SNL). A second group, not receiving the SNL (Sham) was employed as a behavioral control. All rats were then allowed to self-administer various doses of nicotine (0, 3, 10, 30 µg/rat/0.1ml infusion) under both a fixed (FR-3) and a progressive ratio (PR) schedule of reinforcement. Results showed an inverted U-shaped dose-response curve in both groups. However, SNL rats showed an increased nicotine-reinforced lever pressing than Sham on FR-3 when the higher nicotine dose was offered. Furthermore, a

markedly increased motivation to obtain nicotine was observed in SNL rats at the 30 µg/rat dose as assessed on PR schedule while break point at lower dosages was not modified. Initial experiments suggested that this increased motivation to obtain nicotine was probably not due to nicotine's analgesic properties, as sensitivity to von Frey filaments, a measure of mechanical allodynia, was equal before and after the nicotine self-administration session. These data suggest that high dosages of nicotine may be perceived as less aversive in rats with neuropathic pain. Current experiments are aimed at further investigating whether the observed increased nicotine intake and motivation is due to the antinociceptive properties of nicotine that may serve as a coping strategy for the pain, or due to pain-induced neuroadaptations that may ultimately lead to increased vulnerability to nicotine addiction. The behavioral paradigm described here, in which animals with chronic pain increase nicotine self-administration may serve as a unique tool to explore neurobiological substrates of both neuropathic pain and nicotine reinforcement.

Disclosures: A. Cippitelli: None. **J. Schoch:** None. **D. Mercatelli:** None. **J. Wu:** None. **K. Gaiolini:** None. **L. Toll:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.18/MM2

Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA Grant R01DA021754 (JM)

Title: Stress and immediacy effects on reward anticipation

Authors: *L. COSAND[1,2], D. CLEWETT[2], K. VEKARIA[2], K. REYES[2], X. CORDOVA[2], J. MONTEROSSO[2];
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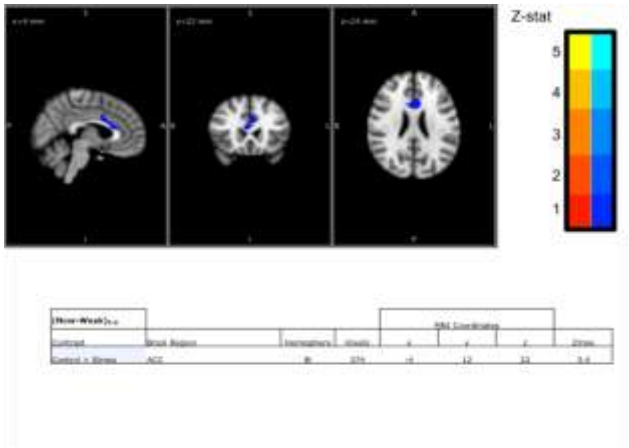
Abstract: Compulsive reward-seeking, seen in addiction and related disorders, has been linked to stress in animal models as well as human populations. One purported mechanism is an increased sensitivity to a reward's incentive salience (motivational strength). Incentive salience is also sensitive to a reward's immediacy, and stress-induced myopia may enhance this effect. The present study hypothesized that subjective stress would increase fMRI BOLD signal in brain regions associated with reward anticipation. Further, this hypothesized stress effect was predicted to interact with reward immediacy such that more dramatic increases were expected during anticipation of immediate rewards than during anticipation of rewards delayed by one week. To test these hypotheses, neuroimaging and behavioral data were collected while 17

overnight-abstinent male cigarette smokers engaged in a reward anticipation task. During the task, which was an adaptation of the monetary incentive delay paradigm (MID; Knutson, Westdorp, Kaiser, & Hommer, 2000), participants attempted to win prizes varying in immediacy (now or in seven days) and type (money or nicotine vapor). When a participant won immediately-available nicotine vapor, he was allowed draw one puff of nicotine vapor before the subsequent trial began. Participants were scanned twice; once after stress induction using the cold pressor task (Lovallo, 1975) and once after a control task. Reward immediacy was associated with faster reaction times (RT) and stronger blood oxygen level-dependent (BOLD) signals in the bilateral insula and anterior cingulate cortices. Under greater stress, however, the immediacy-related anterior cingulate BOLD signal was significantly lower than in the control condition. Furthermore, slower RTs in the stress condition suggested that stress decreases incentive salience of anticipated rewards. These results were unexpected, and may be related to research findings that link anhedonia, addiction, and stress.



Disclosures: L. Cosand: None. **D. Clewett:** None. **K. Vekaria:** None. **J. Monterosso:** None. **K. Reyes:** None. **X. Cordova:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.19/MM3

Topic: C.18. Drugs of Abuse and Addiction

Support: This project was supported by The American Diabetes Association (7-12-BS-135) and The National Institute of Drug Abuse (R24-DA029989 and R25-DA033613).

Title: Enhanced rewarding effects of nicotine in diabetic rats

Authors: *J. A. PIPKIN[1], J. JUARDO[1], L. NATIVIDAD[1], L. CARCOBA[1], A. NAZARIAN[2], L. O'DELL[1];
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Abstract: Epidemiological studies have demonstrated that patients with diabetes display greater vulnerability to tobacco use. However, it is presently unclear if they experience greater rewarding effects of nicotine. To examine this question, we compared the rewarding effects of nicotine in diabetic and healthy control rats using place conditioning (Experiment 1) and intravenous self-administration (IVSA; Experiment 2) procedures. Briefly, diabetes was induced using systemic administration of streptozotocin (STZ), a drug that is toxic to insulin-producing cells in the pancreas. As a result, this drug produces hyperglycemia in rats. Experiment 1 compared place preference in diabetic and healthy control rats that received various doses of nicotine (0, 0.1, 0.2, 0.4, 0.6 mg/kg, SC) in one of two distinct compartments of our conditioning apparatus. Experiment 2 compared IVSA behavior in diabetic and healthy control rats that performed operant responses for increasing doses of nicotine (0.03, 0.06, 0.09 mg/kg/0.1 mL infusion) on a fixed ratio-1 schedule of reinforcement. Nicotine intake was compared in the same rats prior to and following STZ administration. The results from Experiment 1 revealed that diabetic animals displayed a more robust place preference produced by nicotine as compared to healthy controls. Consistent with this, Experiment 2 revealed that diabetic animals displayed a dose-dependent increase in nicotine intake that was higher than healthy controls. In conclusion, the results from both studies revealed that diabetic rats display increased rewarding effects of nicotine as compared to healthy controls. These results suggest that enhanced rewarding effects of nicotine may contribute to greater vulnerability to tobacco use in persons afflicted with diabetes.

Disclosures: J.A. Pipkin: None. **J. Juardo:** None. **L. Natividad:** None. **L. Carcoba:** None. **A. Nazarian:** None. **L. O'Dell:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.20/MM4

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH grant DA012844

Title: Nine generations of selection for high nicotine intake in outbred sprague dawley rats

Authors: *T. NESIL[1], L. KANIT[2], S. POGUN[2], M. D. LI[1];
[1]Psychiatry and NB Sci., Univ. of Virginia, Charlottesville, VA; [2]Ctr. for Brain Res., Ege Univ., Izmir, Turkey

Abstract: Previous animal studies have revealed significant involvement of genetics in nicotine intake; however, the extent of the genetic contribution to this behavior has not been well addressed. Here, we report the first study of nine generations of selection for high and low voluntary nicotine intake in outbred Sprague Dawley rats. Repeated bidirectional mass selection resulted in progressively greater nicotine consumption in the high responder group (or called nicotine-preferring group) but not progressively decreased nicotine intake in the low or non-nicotine responder group (or called nicotine non-preferring group). Our estimated realized heritability for high voluntary nicotine intake is 0.25 vs. close to zero for low voluntary nicotine intake. No response to selection for low or non-nicotine intake behavior could be explained by selecting a not-expressed phenotype, as these animals consumed small amounts of nicotine. These selected lines may provide useful animal models for identifying susceptibility genes and variants for controlling high voluntary nicotine intake in rodents, although we recognize that more generations of selection may be needed to fully realize its power.

Disclosures: T. Nesil: None. **L. Kanit:** None. **S. Pogun:** None. **M.D. Li:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.21/MM5

Topic: C.18. Drugs of Abuse and Addiction

Support: Supported in part by the Intramural Research Program of the National Institute on Drug Abuse, NIH, DHHS

Title: Blockade of nicotine and cannabinoid reward and reinstatement by a cannabinoid CB1-receptor inverse agonist/antagonist and a CB1-receptor neutral antagonist in squirrel monkeys

Authors: S. R. GOLDBERG[1], G. H. REDHI[1], *A. MAKRIYANNIS[2], J. BERGMAN[3], Z. JUSTINOVA[1];
[1]Preclinical Pharmacol. Section, NIDA, IRP, NIH, DHHS, Baltimore, MD; [2]Ctr. Drug Discovery, Northeastern Univ., BOSTON, MA; [3]Preclinical Pharmacol. Lab., McLean Hospital, Harvard Med. Sch., Belmont, MA

Abstract: Nicotine, the main psychoactive component of tobacco, and delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana, play major roles in tobacco and marijuana dependence by acting directly as reinforcers of drug-seeking and drug-taking behavior. Drugs that act as inverse agonist/antagonists at cannabinoid CB1 receptors in the brain attenuate the rewarding and addictive effects of nicotine in rats and of THC in squirrel monkeys, but their clinical use is hindered by potentially serious emotional side effects. Recently developed neutral CB1-receptor antagonists may provide an alternative approach to the treatment of nicotine and cannabinoid dependence. Here we compare attenuation of nicotine and THC reward and reinstatement in squirrel monkeys by the CB1-receptor inverse agonist/antagonist rimonabant and by the recently developed neutral CB1-receptor antagonist AM4113. Both rimonabant and AM4113 reduced two behavioral effects of nicotine and THC that play major roles in tobacco and marijuana dependence: 1) maintenance of high rates of drug-taking behavior, and 2) drug- or cue-induced reinstatement of previously extinguished drug-seeking behavior (relapse). By contrast, neither rimonabant nor AM4113 modified cocaine-reinforced or food-reinforced operant behavior when cocaine or food was available under similar conditions. These findings point to neutral CB1-receptor antagonists as a new class of medications for treatment of both tobacco and marijuana dependence.

Disclosures: S.R. Goldberg: None. **G.H. Redhi:** None. **A. Makriyannis:** None. **Z. Justinova:** None. **J. Bergman:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.22/MM6

Topic: C.18. Drugs of Abuse and Addiction

Support: ANR-2010-BLAN-1439-01

Title: Behavioral effects of cannabinoid-1 receptor agonist in the bed nucleus of the stria terminalis depends on the stage of voluntary nicotine self-administration in rats

Authors: *S. CAILLE[1], M. CADOR[1], F. GEORGES[2], A.-R. REISIGER[1];
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Abstract: We have previously shown that the synaptic potentiation of the network including the infralimbic cortex, bed nucleus of the stria terminals (BNST) and ventral tegmental area is

important for the associative learning process underlying nicotine intravenous self-administration (IVSA) in rats and depends on activation of cannabinoid-1 (CB1) receptors in the BNST. However, it is unknown whether the role of CB1 receptors depends on the level of learning and/or level of nicotine intake. Therefore, we examined the behavioral implication of BNST CB1 receptors at different stages of nicotine IVSA.
In group 1, the CB1 agonist WIN55,212-2 (WIN55) was administered before each session of the first 6 days of acquisition after which IVSA was continued to test for any long-term effects of WIN55. In group 2, the effect of intra-BNST WIN55 was tested after 5 weeks of nicotine IVSA on maintenance and motivation to nicotine. In group 3, 1 week of extinction training was performed after 5 weeks of nicotine IVSA and WIN55 effects were tested on reinstatement.
In group 1, we found that BNST CB1 receptors stimulation impairs transiently the first stage of nicotine IVSA learning mainly due to acute reduced locomotion. This effect disappeared as soon as the treatment ceased. However, later on, WIN55 pretreated rats were unable to adapt to increasing workload to obtain nicotine, both during day to day increased fixed ratio and during progressive ratio schedule of reinforcement. Hence, this early WIN55 pre-treatment prevents later nicotine induced reinstatement while leaving nicotine-associated cue induced reinstatement intact.
In group 2, we observed that stimulation of CB1 receptors in the BNST after 5 weeks of nicotine IVSA does not affect operant responding during fixed ratio and progressive ratio schedule of reinforcement.
In group 3, WIN55 injection blocks both cue-induced and nicotine-induced reinstatement in extinction conditions.
These data imply that CB1 receptor stimulation in the BNST before long-term exposure to nicotine induces protracted behavioral inflexibility and decreased motivation, without altering nicotine intake. Moreover, rats treated with WIN55 during acquisition phase of nicotine IVSA are still sensitive to the reward-associated cue, but not to nicotine itself. However, after a history of nicotine, while CB1 agonist in the BNST does not affect motivation to nicotine and nicotine-taking, it blocks the incentive properties of both nicotine and nicotine-paired stimulus.

Disclosures: S. Caille: None. **M. Cador:** None. **F. Georges:** None. **A. Reisiger:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.23/MM7

Topic: C.18. Drugs of Abuse and Addiction

Title: Predictors of drug-seeking behavior in rodent reinstatement models of relapse

Authors: *S. M. GOEBEL-GOODY, E. DUNN-SIMS, D. B. HORTON, A. ROSADO, A. FOOTE, C. TYSZKIEWICZ, J. K. DASILVA, N. NAWREEN, A. N. MEAD;
Global Safety Pharmacol., Pfizer Inc., Groton, CT

Abstract: Reinstatement of drug-seeking behavior in rats previously trained to self-administer drugs of abuse is widely used for studying relapse to addiction. There is considerable face and criterion validity for the reinstatement model, given that conditions which induce reinstatement in rats (e.g., exposure to drug, associated environmental cues, or stressors) also provoke relapse in humans. Even so, the extent of reinstatement can vary depending on the conditions used to induce reinstatement (e.g., cues only, prime only or combined cues and prime), as well as various factors during training (e.g., drug training dose, presence of secondary auditory reinforcers such as clickers or tones). Oftentimes, individual rats also show variability in the magnitude of the reinstatement response. These findings pose the question as to whether there are specific endpoints during drug self-administration training or the subsequent extinction phase that may predict reinstatement of drug-seeking behavior. In an initial study, rats were trained to respond for intravenous nicotine (0.015 mg/kg/infusion) under a fixed ratio schedule of reinforcement. We analyzed whether the extent of each animal's response (i.e., number of active lever presses) under a combined nicotine cue- and prime-induced reinstatement condition directly correlated with the following endpoints obtained from the last two self-administration training sessions: average number of infusions, average active lever presses, and average selectivity for the active lever over the inactive lever. Infusion stability for the last two sessions, number of active lever presses on the first extinction session, and total number of sessions required for training were also examined. Of these, only the average number of infusions was significantly correlated with the reinstatement response, where infusions were positively correlated with the number of active lever presses during reinstatement ($r = 0.31$, $P < 0.005$). These results indicate that rats with the highest reinstatement also had the greatest drug intake during the last two self-administration sessions. A similar positive correlation was observed in studies with higher training doses of nicotine (0.03 mg/kg/infusion) ($r = 0.64$, $P < 0.0001$). Together, these findings suggest that session drug intake (measured by number of infusions) during self-administration training may be particularly important for predicting reinstatement of nicotine-seeking behavior. Further studies will determine whether this direct correlation persists across different reinstatement conditions and drugs of abuse, such as fentanyl.

Disclosures: S.M. Goebel-Goody: A. Employment/Salary (full or part-time):; Pfizer, Inc. **E. Dunn-Sims:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **D.B. Horton:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **A. Rosado:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **A. Foote:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **C. Tyszkiewicz:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **J.K. DaSilva:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **N. Nawreen:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **A.N. Mead:** A. Employment/Salary (full or part-time):; Pfizer, Inc..

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.24/MM8

Topic: C.18. Drugs of Abuse and Addiction

Title: Attenuating combined cue and prime-induced reinstatement of nicotine-seeking using the novel D3-receptor antagonist PF-04363467

Authors: *E. R. DUNN-SIMS[1], A. SAWANT-BASAK[2], M. VANASE-FRAWLEY[1], A. ROSADO[1], C. STEPPAN[1], N. STRATMAN[2], C. TYSZKIEWICZ[1], T. WAGER[2], A. MEAD[1]; [1]Pfizer Inc, Groton, CT; [2]Pfizer, Cambridge, MA

Abstract: Learned associations between the rewarding properties of drugs of abuse and environmental cues contribute to craving and relapse in humans. Dopamine D3-receptors are preferentially expressed in mesocorticolimbic DA projection areas: areas of the brain highly associated with reward-related learning induced by drugs of abuse. Nicotine dependence is a chronic relapsing disorder and increasing evidence supports a model for which D3-receptor activity is implicated in the relapse-related cellular and behavioral effects underlying nicotine-seeking behavior. In the past, pharmacological studies aimed at differentiating the role and contribution of D3 receptors in nicotine-dependence was limited by the lack of D3-selective compounds. The principle purpose of this study was to determine whether the novel D3-receptor antagonist PF-04363467, and the prototype D3 antagonists GSK598809 and SB277011-A, could attenuate reinstatement of nicotine-seeking in rats under different test conditions. Male Sprague-Dawley rats were initially trained to self-administer nicotine, I.V., under a fixed ratio schedule of reinforcement using a standard 2-lever choice design, with infusion-paired cues. Following acquisition, an extinction period commenced to dissociate the act of lever pressing from delivery of nicotine. Reinstatement tests were then conducted using a within-subjects design, with reinstatement induced by both a nicotine prime and a combination nicotine prime and cue. For reinstatement tests, animals were treated with PF-04363467, GSK598809 or SB277011-A prior to each session. Congruent with the literature implicating D3-receptors with the rewarding/reinforcing effects of drugs, and specifically the association between drugs of abuse and drug-associated environmental stimuli, the present findings suggest D3-receptor antagonists can attenuate reinstatement of nicotine seeking in rats under certain test conditions. The effect of these antagonists in blocking reinstatement of nicotine-seeking behavior was compared to their affinity to bind to, and occupy, the dopamine D3-receptor. Taken together, these observations

support the potential use of novel D3-receptor antagonists in preventing nicotine-relapse and craving in humans.

Disclosures: E.R. Dunn-Sims: None. **A. Rosado:** None. **C. Tyszkiewicz:** None. **A. Mead:** None. **C. Steppan:** None. **M. Vanase-Frawley:** None. **T. Wager:** None. **A. Sawant-Basak:** None. **N. Stratman:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.25/MM9

Topic: C.18. Drugs of Abuse and Addiction

Support: DA027840

Title: D1 dopamine antagonist treatment with SCH 23390 to decrease nicotine self-administration in rats

Authors: S. SLADE, C. WELLS, *R. D. SCHWARTZ-BLOOM, A. H. REZVANI, J. E. ROSE, E. D. LEVIN;
Duke Univ. Med. Ctr., DURHAM, NC

Abstract: Dopaminergic systems in the brain have long been known to be critically involved with drug addiction in general and nicotine addiction in particular. However, D2 antagonist treatment has not been found to effectively reduce nicotine self-administration. In fact the D2 antagonist haloperidol has been shown to increase smoking. The other principal subtype of dopamine receptors, the D1 receptor, has not been as extensively investigated. Adult female Sprague-Dawley rats were trained to self-administer nicotine IV at 30 µg/kg/infusion. Acute SC doses of 10-50 µg/kg of the D1 antagonist SCH 23390 significantly reduced nicotine self-administration but this dose range also significantly reduced food motivated responding (N=6). However, a lower dose range of SCH 23390 (1.25-5 µg/kg) was found to significantly reduce nicotine self-administration without a significant effect on food motivated responding (N=20). Interestingly, there was the appearance of a non-monotonic, dose-effect function in this lower dose range of 1.25-5 µg/kg with a significant quadratic effect over that range with a slight increase in nicotine self-administration at the lowest dose tested (1.25 µg/kg). A critical locus of action for D1 antagonist effects may be the insular cortex. We have found that local infusion of SCH 23390 into the insular cortex significantly decreased nicotine self-administration. This study provides information supporting the involvement of D1 receptor systems in the control of

nicotine self-administration and the efficacy of systemic administration. D1 antagonists hold promise of as potential treatment for smoking cessation.

Disclosures: S. Slade: None. **C. Wells:** None. **R.D. Schwartz-Bloom:** None. **A.H. Rezvani:** None. **J.E. Rose:** None. **E.D. Levin:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.26/MM10

Topic: C.18. Drugs of Abuse and Addiction

Support: DA027990

Title: The α4β2 nicotinic desensitizing agent VMY 2-109 significantly decreases nicotine self-administration in rats

Authors: *E. D. LEVIN[1], S. SLADE[2], C. WELLS[2], S. A. BRIGGS[2], G. ZHANG[2], A. H. REZVANI[2], Y. XIAO[3], K. J. KELLAR[3], V. M. YENUGONDA[3], M. PAIGE[3], M. BROWN[3]; [1]Duke Univ. Med. Ctr., DURHAM, NC; [2]Psychiatry, Duke Univ., Durham, NC; [3]Georgetown Univ., Washington, DC

Abstract: Nicotinic receptor desensitization holds promise as an effective treatment of tobacco addiction. Previously, we found that sazetidine-A which desensitizes α4β2 nicotinic receptors of all configurations and is an agonist at the α4(2) β2(3) receptor configuration significantly decreased nicotine self-administration in rats. We also found that its analog VMY-2-95 likewise significantly decreased nicotine self-administration. In the current study we tested another compound VMY 2-109 with similar effects, desensitizing α4β2 nicotinic receptors. Young adult female Sprague-Dawley rats were fitted with IV catheters and were trained to self-administer nicotine (0.03 mg/kg/infusion) on a fixed ratio 1 schedule for ten sessions. VMY-2-109 was administered SC ten minutes before the start of 45-minute sessions in a counterbalanced design which was conducted two times. VMY-2-109 at 3 mg/kg significantly ($p<0.025$) reduced nicotine self-administration. This is the same effective dose as previously seen with sazetidine-A and VMY-2-95. There was a significant ($p<0.05$) interaction of VMY-2-109 x baseline level of nicotine self-administration (below or above the median level of self-administration during pre treatment baseline training). The reduction in nicotine self-administration caused by acute VMY-2-109 was particularly evident ($p<0.0005$) in rats with high pre-treatment baseline levels of nicotine self-administration (N=14) but not in those with low baseline levels (N=15). Food motivated responding was significantly decreased by both the 1 and 3 mg/kg acute doses of

VMY-2-109, indicating that the effect was not specific to nicotine. We have not yet tested it on other drugs of abuse, but previously we have found that sazetidine-A significantly reduces self-administration of a variety of drugs of abuse including alcohol, cocaine and methamphetamine. The reduction in nicotine self-administration by VMY-2-109 did not appear to be due to sedative effects. Like VMY-2-95, VMY-2-109 caused significant increases in activity at all three doses tested 0.3, 1 and 3 mg/kg. These studies show that acute VMY-2-109 reduces nicotine self-administration at a dose level that does not cause sedative effects. VMY-2-109 holds promise as a novel aid for smoking cessation and should be further studied to determine its safety and efficacy for being used as a novel aid for smoking cessation.

Disclosures: E.D. Levin: Other; Patent pending, Georgetown University, Duke University. **S. Slade:** None. **C. Wells:** None. **S.A. Briggs:** None. **G. Zhang:** None. **A.H. Rezvani:** Other; Patend Pending, Duke University. **Y. Xiao:** Other; Patent Pending, Georgetown University. **K.J. Kellar:** Other; Patent pending, Georgetown University. **V.M. Yenugonda:** Other; Patent pending. **M. Paige:** Other; Patent pending, Georgetown. **M. Brown:** Other; Patent pending, Georgetown University.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.27/NN1

Topic: C.18. Drugs of Abuse and Addiction

Support: DA10588

DA026600

Title: Effects of methamphetamine and nicotine on the habituation of reinforcing effectiveness of sensory stimuli

Authors: *D. R. LLOYD, J. B. RICHARDS;
Res. Inst. On Addictions, Buffalo, NY

Abstract: Background:
Visual stimuli (VS) determined to have primary reinforcing effects are classified as sensory reinforcers to differentiate them from other more biologically important primary reinforcers (e.g. food and water). VS are weak reinforcers and their reinforcing effects rapidly habituate. The psychomotor stimulants methamphetamine (METH) and nicotine (NIC) increase the reinforcing effectiveness of VS. We examined the effects of METH and NIC on the habituation of the

reinforcing effectiveness of VS.
Methods:
The effects of saline (n = 10), NIC (0.40 mg/kg, n = 10) and METH (0.75 mg/kg, n = 9) on habituation of reinforcing effectiveness were evaluated. Phase 1 (10 sessions) examined the operant level of responding, phase 2 (10 sessions) examined the effects of drug on the operant level of responding, and phase 3 (5 sessions) examined the combined effects of drug and a response contingent VS on the rate of responding. In all three phases within-session changes in the reinforcing effectiveness of VS were examined by plotting and analyzing responding in successive 8 min epochs of each 40 minute test session.
Results:
Nicotine decreased habituation rate and did not affect response rate, while METH decreased habituation rate and increased response rate. Introduction of a novel VS decreased habituation rate and increased responding.
Conclusions:
Habituation of reinforcer effectiveness may modulate operant responding for sensory reinforcers and may play a role in the effects of stimulant drugs on operant behavior.

Disclosures: D.R. Lloyd: None. **J.B. Richards:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.28/NN2

Topic: C.18. Drugs of Abuse and Addiction

Title: Adolescent methylphenidate exposure increases the reinforcement enhancing effects of nicotine

Authors: *D. PETERSON, A. B. SHEPPARD, M. I. PALMATIER, R. BROWN;
East Tennessee State Univ., Johnson City, TN

Abstract: Methylphenidate (MPH) is widely prescribed during childhood and adolescence for treatment of attention deficit and hyperactivity disorder. MPH is also one of the most commonly abused prescription drugs. However, the effects of MPH exposure and MPH abuse on incentive motivation are not well known. Moreover, MPH abuse during adolescence could increase sensitivity to the incentive motivational effects of other abused drugs such as nicotine in adulthood. Thus, the goals of this experiment were to investigate the effects of MPH exposure on the motivation to obtain sucrose during adolescence and to examine whether adolescent

methylphenidate exposure altered the incentive motivational effects of nicotine (NIC) in adulthood. Incentive motivation was measured using an operant conditioning paradigm with sucrose available under a progressive ratio schedule of reinforcement (PR). Adolescent female rats were used because our previous studies have shown stronger sensitization to the locomotor stimulant effects of MPH. Rats arrived at post-natal day 21 (P21) and were shaped to respond for sucrose (20% w/v) on the PR schedule beginning on P24. After stable operant responding was established, rats were randomly assigned to receive either MPH (n=7) or SAL (n=6) injections (intraperitoneal) 30 min prior to test sessions, with the constraint that sucrose rewards earned did not differ between groups. Injection tests began on P36 and were carried out on alternating days for 10 total tests (P36-54). Although there was a trend for increased motivation for sucrose in the MPH group, it did not reach statistical significance. No further testing occurred until the rats reached adulthood (P55-P78). Over the next 5 days (P79-P84), all rats were pretreated with subcutaneous NIC injections (0.4 mg/kg base) 15 min before testing sessions. Following this initial 'sensitization' period, rats were tested with different NIC doses (0-1 mg/kg base) from P85-P92. During the sensitization period, NIC increased responding equally in both groups. However, during the dose-response testing, rats in the MPH group were more sensitive to the incentive motivational effects of NIC - the median effective dose was significantly lower for rats exposed to MPH in adolescence. The findings suggest that MPH may have limited reinforcement enhancing effects in adolescents. However, exposure to MPH during adolescents may increase the incentive motivational effects of NIC in adulthood.

Disclosures: D. Peterson: None. **A.B. Sheppard:** None. **M.I. Palmatier:** None. **R. Brown:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.29/NN3

Topic: C.18. Drugs of Abuse and Addiction

Support: Johnson Center for Cancer Research

Title: Nicotine exposure during acquisition increases the motivational valence of non-drug reinforcers

Authors: A. B. SHEPPARD[1], R. M. FLOYD[2], Z. DIETZ[2], *M. I. PALMATIER[1];
[1]East Tennessee State Univ., Johnson City, TN; [2]Psychology, Kansas State Univ., Manhattan, KS

Abstract: Nicotine (NIC) has dual effects on reinforcement - both increasing responding for NIC as well as increasing motivation to approach and engage reward-associated cues. We have hypothesized that NIC alters incentive learning increasing the value of non-drug stimuli associated with rewards. We examined whether NIC given during acquisition of responding for a visual stimulus (VS; extinction of all lights in chamber for 30 s) increased the motivation to obtain the VS and whether the VS acquired more motivation value when responding for the VS was acquired under the influence of NIC. To do so, we determined if changes in responding during acquisition would persist beyond the pharmacological effects of NIC. In Experiment 1, rats were randomly assigned to receive either NIC (Acq-NIC group) or saline (Acq-SAL group) injections prior to acquisition of responding for the VS. Rats received designated injections 15 minutes prior to responding for the VS on fixed ratio 1 (FR1) and FR3 schedules of reinforcement. During the final tests, all rats receive SAL injections prior to responding under an FR3 and were subsequently shifted to a progressive ratio (PR) reinforcement schedule - a more sensitive measure of motivation. Under the PR schedule, rats in the Acq-NIC group had higher breaking points than the Acq-SAL group, even though both groups were pretreated with SAL. Experiment 2 used similar procedures but instead of a SAL group, rats were assigned to a Post-NIC group to equate total drug exposure. This group received NIC injections in the home cage 3 hrs after test sessions were completed. The Pre-NIC group received NIC injections 15 min before all acquisition sessions. During acquisition, rats were shifted from an FR3 to a PR schedule. Once stability criteria were met on the PR, all rats received placebo injections 15 min before test sessions. Even though both groups were pretreated with SAL during these tests, the Pre-NIC group had significantly higher breaking points than the Post-NIC group. Follow-up studies used a more potent reinforcer (sucrose) in order to increase the value of incentives in the test chamber. The findings suggest that NIC can potently increase incentive learning.

Disclosures: A.B. Sheppard: None. **M.I. Palmatier:** None. **Z. Dietz:** None. **R.M. Floyd:** None.

Poster

545. **Nicotine: Reinforcement, Seeking, and Reinstatement**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 545.30/NN4

Topic: C.18. Drugs of Abuse and Addiction

Support: R01 DA10464

Title: Nicotine enhances the rewarding properties of sucrose

Authors: *R. SCHASSBURGER[1,2], L. E. RUPPRECHT[1,2], T. T. SMITH[3], D. M. BUFFALARI[1,2], E. THIELS[4,2,5], E. C. DONNY[3], A. F. SVED[1,3,2];
[1]Neurosci., [2]Ctr. for Neurosci., [3]Psychology, [4]Neurobio., [5]Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Nicotine serves not only as a primary reinforcer but also acts to enhance the reinforcing properties of environmental and non-pharmacological stimuli. Nicotine has been shown to enhance the reinforcing properties of a mildly-reinforcing visual stimulus and to promote operant responding. Reinforcement-enhancement occurs irrespective of whether nicotine delivery is contingent or non-contingent on behavior and experimenter- or self-administered. The present study sought to evaluate the nicotine reinforcement-enhancement effect on a natural reward. Adult male rats (n = 32) were allowed to nosepoke for sucrose (45 mg pellets) on a progressive ratio (PR) schedule of reinforcement. Sucrose maintained a high level of responding at the active nosepoke port. Nicotine (0.5 mg/kg; s.c.) administered 10 min prior to the operant session significantly increased sucrose pellets earned by 22% ($p<0.01$) and active responses by 99% ($p<0.01$), without increasing responding into the inactive nosepoke ($p>0.05$). This selective effect of nicotine on responding for sucrose was observed on the first day of nicotine treatment, and was maintained over six sessions of daily nicotine treatment. Blockade of nicotinic acetylcholine receptors (nAChR) by systemic administration of mecamylamine (3.0 mg/kg; s.c.) immediately prior to the daily nicotine treatment reduced responding for sucrose to the same level of responding as observed in the absence of nicotine. Systemic administration of dopamine antagonists (1, 3, 10, or 30 μg/kg SCH23390; 1, 3, 10, or 30 μg/kg eticlopride; s.c.) also reduced the number of sucrose pellets earned; ongoing studies are investigating the specific involvement of dopamine receptors in the nucleus accumbens in nicotine reinforcement-enhancement. These results demonstrate that the robust enhancement of environmental stimuli by nicotine in the maintenance of operant behavior extends to natural rewards. Moreover, this effect may in part be mediated by nicotinic acetylcholine receptors and dopamine receptors. Our finding that nicotine enhances responding for a food reward is particularly interesting in light of the anorexigenic effects of nicotine. It underscores the robustness of the reinforcement-enhancement effect of nicotine, and suggests that nicotine's reinforcement-enhancement can overcome competing consequences of nicotine.

Disclosures: R. Schassburger: None. **L.E. Rupprecht:** None. **T.T. Smith:** None. **D.M. Buffalari:** None. **E. Thiels:** None. **E.C. Donny:** None. **A.F. Sved:** None.**Poster**

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.01/NN5

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA032856

NIH Grant DA012677

Title: Preferences for cocaine and food rewards: Neurochemical profiles in the nucleus accumbens distinguish cocaine and pellet preferring rats

Authors: *A. N. PERRY[1], C. WESTENBROEK[1], L. JAGANNATHAN[1], J. B. BECKER[1,2,3,4]; [1]The Mol. & Behavioral Neurosci. Inst., [2]Dept. of Psychology, [3]Dept. of Psychiatry, [4]Neurosci. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Given the opportunity to self-administer either palatable food pellets or cocaine, most rats choose pellets. We have previously shown that cocaine-preferring (CP) rats display increased drug intake at the expense of earning pellets, have enhanced motivation for cocaine and reduced motivation for pellets and show greater reinstatement of drug seeking following cocaine priming and exposure to a drug-paired cue. We propose that CP rats represent an addictive phenotype that distinguishes them from the majority of rats that are able to regulate their drug intake and maintain a pellet preference (PP). The objective of the current study was to characterize the cocaine-induced dopamine (DA) and norepinephrine (NE) responses in the nucleus accumbens (NAc) of PP and CP rats. Male and female Sprague-Dawley rats were fitted with jugular catheters and microdialysis guide cannulae targeting the NAc. Rats self-administered cocaine (0.4 mg/kg/inf) and pellets (45 mg, BioServ) on a fixed ratio 5 schedule 4 days a week for 7 weeks to identify PP and CP individuals. Motivation for cocaine and pellets was assessed once each week on a progressive ratio schedule. The DA and NE responses to an acute cocaine infusion (1.2 mg/kg, i.v.) were investigated both early and late during self-administration. When response preferences were stable late during self-administration, rats were treated with each of the following: the beta adrenergic receptor antagonist S-(-)-propranolol (10 mg/kg, i.v.), the alpha 1 adrenergic receptor antagonist terazosin (2 mg/kg, i.v.) or the saline vehicle (1 ml/kg, i.v.) 15 minutes before self-administration in a counter-balanced order to examine the roles of noradrenergic receptors in preference behavior. PP and CP rats had similarly robust DA and NE responses in the NAc early in self-administration. Preliminary results indicate that CP rats displayed attenuated DA and NE responses late in self-administration when preferences were stable, whereas PP rats maintained a normal DA response and an attenuated NE response. Propranolol reduced both cocaine and pellet intake in CP rats, with less pronounced effects in PP rats. Terazosin's effects were mixed. In conclusion, the expression of a preference for cocaine is associated with a specific neurochemical profile distinct from animals that do not prefer cocaine. The results of pharmacological manipulations indicate the potential for interaction between the DA and NE systems in the display of preference behavior.

Disclosures: A.N. Perry: None. **C. Westenbroek:** None. **L. Jagannathan:** None. **J.B. Becker:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.02/NN6

Topic: C.18. Drugs of Abuse and Addiction

Support: FMKirby Fdn

Center for Advanced Technololgy CUNY

BRODERICK BRAIN Fdn

McKenzie Fdn

Title: Cocaine affects acoustic startle in concert with estrus cycle stages

Authors: *L. B. MALAVE[1,2,3], P. A. BRODERICK[1,2,3,4];
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Abstract: Startle response to auditory stimuli via prepulse inhibition (PPI) examines the ability to neuronally gate the sensory/motor network to heed warning signals. PPI refers to the process in which a weaker prestimulus (prepulse) impedes the reaction of a subject to a subsequent strong startling stimulus (pulse). PPI is significantly decreased in cocaine-administered human subjects (Geyer; Braff, 1987). Moreover, we reported sex differences to acoustic startle response during cocaine-administration (Broderick and Rosenbaum, 2013). There are many studies showing that sensitivity to cocaine reward varies during the estrous cycle (Zakharova et al., 2009; Kippin et al., 2005; Feltenstein et al., 2009). Furthermore, we have published a body of evidence on cocaine induced estrus cycle changes. Thus, these changes in estrus cycle may be the underlying cause of the sex differences in acoustic startle response for cocaine-administered animals. In addition, caffeine did not affect estrus cycle nor does the combination of cocaine and caffeine. We observed that caffeine blocks the cocaine induced estrus cycle changes and may neuro-protect against the decrease in PPI when administered in concert with cocaine (Broderick et al., 2012). In the present study we set out to correlate estrus cycle stages with acoustic startle responses on varying doses of cocaine, caffeine and combination of both.

Disclosures: L.B. Malave: None. **P.A. Broderick:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.03/NN7

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA06227

Title: SIRT1 and MAOA regulation in cocaine excited delirium

Authors: *S. GARAMSZEGI, L. DUQUE, X. XIE, N. ADI, D. C. MASH;
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Abstract: Excited delirium syndrome (ExDS) is a condition associated with cocaine abuse that manifests as a combination of psychomotor agitation, violent and bizarre behavior, extraordinary strength, and hyperthermia. ExDS subjects often die in police custody, with heighted reactivity to restraint stress. SIRT1, a NAD^+-dependent protein deacetylase regulates anxiety and exploratory drive by monoamine oxidase A (MAOA) activation (Libert, S. et al., Cell 147: 1459-72, 2011). Knockout mice lacking functional SIRT1 have lower levels of MAOA and are less susceptible to depression and anhedonia following social defeat. In contrast, SIRT1 overexpressors have higher levels of MAOA. SIRT1 deacetylates transcription factor NHLH2 to increase the activation of the MAOA promoter, resulting in a reduction in serotonin. Gene expression differences in the substantia nigra (SN) were analyzed in cases of cocaine-related ExDS (n=43) and compared to cocaine intoxication deaths (n=108) and age-matched drug-free controls (n=107). In ExDS cases, SIRT1 (1.5-fold; p= 0.0001) and MAOA (2.1-fold; p=0.0147) were upregulated compared to cocaine abusers and control subjects. SIRT1 deacetylates heat shock transcription factor (HSF1) which activates its binding to the HSP70 promoter and increases its transcription under conditions of elevated core body temperature. In ExDS, HSPA1B gene is significantly upregulated (2.5-fold; p <0.0001) in the SN. Genetic variation in SIRT1 has been associated with body mass index (BMI) and risk of obesity. ExDS disproportionately affects male cocaine abusers with high BMI values (OR = 2.8; C.I. 1.2 - 6.4), suggesting an association between food intake patterns and obesity in these subjects. SIRT1 has been suggested to link the nutritional status of animals to behavior. Cocaine abusers decrease food intake during a period of binge use, following by hyperphagia during states of withdrawal. A genetic study of SIRT1 polymorphisms may suggest a role of this sirtuin in the heightened anxiety-like state associated with terminal delirium and hyperthermia in ExDS.

Disclosures: S. Garamszegi: None. **L. Duque:** None. **X. Xie:** None. **N. Adi:** None. **D.C. Mash:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.04/NN8

Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA/IRP

Title: Glia-derived glutamate differentially modulates cocaine-taking and cocaine-seeking behavior in rats

Authors: *Z. XI, H.-J. YANG, X. LI, G.-H. BI, H.-Y. ZHANG;
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Abstract: It is well documented that neuronal glutamate release plays a critical role in cocaine- or cue-induced reinstatement of drug-seeking behavior. However, it remains unclear whether glia-derived glutamate similarly regulates cocaine-taking and cocaine-seeking behavior. In the present study, we evaluated the effects of TBOA, a selective glial glutamate transporter (GLT-1) inhibitor, and NPPB, a glial anion channel blocker, on cocaine self-administration and reinstatement of drug-seeking behavior. We found that: 1) local perfusion of TBOA into the NAc significantly elevated, while NPPB lowered extracellular glutamate in a dose-dependent manner; 2) microinjections of TBOA into the NAc, but not the dorsal striatum, inhibited cocaine self-administration under both fixed-ratio (FR2) and progressive-ratio (PR) reinforcement, while microinjections of NPPB into the NAc had no effect on cocaine self-administration; 3) this effect on cocaine self-administration was blocked by co-administration of TBOA and AP-5 (a NMDA receptor antagonist), ifenprodil or Ro25-6981 (selective NMDA-NR_{2B} receptor inhibitors), but not by DNQX (an AMPA receptor blocker) or NVP-AAM007 (a NMDA-NR_{2A} receptor antagonist), suggesting a NMDA-NR_{2B}-mediated effect; 4) conditioned knockdown of GLT-1 in the NAc by antisense oligonucleotides also decreased cocaine self-administration in rats in a dose-dependent manner; 5) microinjections of TBOA, but not NPPB, into the NAc, but not the dorsal striatum, reinstated drug-seeking behavior in rats extinguished from cocaine self-administration. Taken together, the present findings suggest that elevation of extracellular glutamate in the NAc by inhibiting glial GLT-1 inhibits cocaine-taking behavior in rats during self-administration, and reinstates cocaine-seeking behavior in rats during reinstatement testing.

In contrast, attenuation of glial glutamate release via anion channels has no effect on cocaine-taking and cocaine-seeking behavior. *Supported by NIDA-IRP*.

Disclosures: Z. Xi: None. **H. Yang:** None. **X. Li:** None. **G. Bi:** None. **H. Zhang:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.05/NN9

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH grant 5R01DA023248

NIH grant 5K02DA026990

Title: Thalamic connectivity with independent functional components of cognitive control distinguishes cocaine addicts from healthy controls

Authors: *C.-S. R. LI, S. ZHANG, S. HU;
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Abstract: BackgroundOur recent work combining brain imaging and longitudinal follow-up described deficits in thalamic cortical processes of cognitive control that predicted relapse in cocaine addicts (Luo et al., 2003, Brain). Here, we examined how thalamic connectivity with components of cognitive control, as identified by independent component analysis (ICA), distinguished cocaine addicts from healthy individuals.
MethodsFunctional MRI data were collected on the stop signal task from individuals with cocaine dependence (CD, n = 100) and demographics-matched health controls (HC, n = 100). Preprocessed time series were analyzed with group ICA (GIFT, http://icatb.sourceforge.net/, version 1.3i) to identify spatially independent and temporally coherent networks. After back-reconstruction, z values of each component for each voxel of the whole thalamus were computed as a feature vector for all subjects. Two-sample t tests identified feature vectors that were significantly different between CD and HC. A threshold was applied to this difference to identify final feature sets for classification with a multivariate pattern classifier.
ResultsLeave-one-out cross-validation with a total of 2,285 feature vectors ($p<0.05$, CD vs. HC) distinguishes CD and HC at 95% accuracy. Notably, the thalamus voxels aggregated in specific nuclei such that their functional connectivity with specific IC distinguished CD from HC. For instance, voxels clustered in the medial nucleus for medial prefrontal cortex, motor cortex, and regions of the default mode network as well as in the lateral nucleus for cuneus/precuneus and

subcortical areas to achieve successful classification.
ConclusionsThese findings suggested that, by connecting to distinct cerebral networks and potentially serving different functions in cognitive control, subnuclei of the thalamus played different roles in distinguishing CD from HC. These new findings shed further light on deficits of cognitive control in cocaine dependence and suggest a useful approach to unraveling the neural endophenotypes of complex mental disorders.

Disclosures: C.R. Li: None. **S. Zhang:** None. **S. Hu:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.06/NN10

Topic: C.18. Drugs of Abuse and Addiction

Support: DA 006886

Title: Tracking changes of medium spiny projection neurons in the dorsolateral striatum during chronic cocaine self-administration

Authors: *K. COFFEY, D. J. BARKER, S. MA, J. KULIK, M. O. WEST;
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Abstract: It has been posited that the transition from drug use to addiction, involves a shift from goal-directed drug seeking to habitual drug taking. It is thought that one neural substrate for this shift may be the Dorsal Striatum. It is hypothesized that early in training Dorsomedial Striatum (DMS) processes a (response - outcome) relationship and produces a goal-directed behavior. After extended training, the behavior becomes skilled and the Dorsolateral Striatum (DLS) is thought to produce the skill habitually. However, this model of DLS involvement in addiction is woefully oversimplified. DLS consists of at least 3 cell types, all of which serve different functions. First are the interneurons, Fast-Spiking Interneurons (FSIs - Gabbaergic ~10%) and Tonically Active Neurons (TANs - Cholinergic ~1%), which play critical roles in modulating the activity of projection neurons. Based on their sensory input, and direct connection to projection neurons, it has been suggested that the interneurons are encoding the habitual stimulus response relationship. However, the remaining medium spiny projection neurons (MSNs ~89%) are the most frequently studied in their relationship to habitual behavior. These neurons are somatotopic in nature, similar to motor cortex. They respond in unconditioned phasic bursts during movement of discrete body parts. Often, labs that study these neurons do not account for this specificity, confounding their habit learning results with simple movement differences. To control for motor

differences, this study tracked a single MSN population (head movement neurons) during chronic cocaine self-administration (SA), using a movement operant. By ensuring that cocaine taking responses could be matched and identical across days, we are able to draw conclusions about learning related changes in DLS activity across chronic cocaine self-administration. MSNs in the DLS process discrete motion characteristics (i.e. velocity), and undergo motor-training related changes in activity, that correlate to changes in animal behavior. This evidence indicates that MSNs in DLS serve as an important training signal during the learning of skilled behavior, but they do not undergo the population level increase in activity proposed by the habit hypothesis.

Disclosures: K. Coffey: None. **D.J. Barker:** None. **S. Ma:** None. **J. Kulik:** None. **M.O. West:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.07/NN11

Topic: C.18. Drugs of Abuse and Addiction

Support: K01DA031747

R01 DA22339

Title: Distinct roles of PKC signaling at direct and indirect pathway medium spiny neurons during reinstatement of cocaine-seeking

Authors: *P. I. ORTINSKI, L. A. BRIAND, C. PIERCE, H. D. SCHMIDT;
Univ. of Pennsylvania, PHILADELPHIA, PA

Abstract: Cocaine abuse in humans is frequently characterized by withdrawal from and relapse to chronic drug use. The relapse to cocaine seeking in rodents can be modeled as enhanced behavioral responding following exposure to a pharmacological trigger. In particular, reinstatement of cocaine seeking can be triggered by stimulation of dopamine receptors in the nucleus accumbens, a component of the mesolimbic dopamine reward pathway. Here, we show that microinjection of either D1-like or D2-like dopamine receptor agonists into the rat nucleus accumbens shell leads to reinstatement of cocaine-seeking behavior. Administration of the protein kinase C antagonist, chelerythrine, attenuates reinstatement induced by the D2-like, but not the D1-like dopamine receptor agonists. We explore the possible neuronal mechanisms for this phenomenon by evaluating the effects of chelerythrine at medium spiny neurons of the direct

(D1-expressing) and indirect (D2-expressing) pathways. We find that exposure of brain slices to chelerythrine modulates excitatory and inhibitory synaptic signaling differently at D1- and D2-expressing neurons. Furthermore, sensitivity of evoked synaptic activity to chelerythrine is dependent upon the frequency of stimulation of synaptic afferents. These results suggest the possibility that distinct signaling patterns at neurons of the direct and indirect pathways in the nucleus accumbens shell underlie distinct roles of protein kinase C during reinstatement of cocaine seeking by agonists at D1-like and D2-like dopamine receptors.

Disclosures: P.I. Ortinski: None. **L.A. Briand:** None. **C. Pierce:** None. **H.D. Schmidt:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.08/NN12

Topic: C.18. Drugs of Abuse and Addiction

Support: DA025617

DA035088

DK074734

DA15758

Title: Corticostriatal regulation of glutamate homeostasis by astrocytes in the nucleus accumbens: Effects of pacap on cystine-glutamate exchange by system x_c- in the nucleus accumbens

Authors: *L. KONG[1], A. MADAYAG[3], J. M. RESCH[2], S. CHOI[2], J. R. MANTSCH[2], D. A. BAKER[2];
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Abstract: Repeated cocaine administration is associated with a persistent disruption of glutamate homeostasis in the nucleus accumbens, and this may be due to altered release and/or clearance by astrocytes. Previous work has shown decreased activity of glutamate release from system x_c- and glutamate clearance by GLT-1, and both mechanisms are expressed on astrocytes. The importance of these findings is evident from studies demonstrating that normalizing the function of either mechanism reduces the magnitude of cocaine-primed reinstatement. The purpose of these studies was to explore the potential involvement of the pituitary adenylyl

cyclase activating polypeptide (PACAP) in cocaine-induced blunting of system xc- activity. Our interest in this peptide stems from earlier observations that it likely originates from corticostriatal projection neurons since PACAP is co-localized with VGLUT in the prefrontal cortex. This is important because cocaine appears to reduce the activity of corticostriatal projections. In addition, PAC1R, the primary receptor for PACAP is expressed on astrocytes. Lastly, PAC1R signaling has been shown to regulate glutamate clearance by astrocytes. To examine the potential for PACAP to regulate system xc-, we first examined the impact of PAC1R signaling on cystine uptake in rat cortical and striatal cultures. We found that 24 hr PACAP treatment produced a concentration-dependent increase in ^{14}C-cystine uptake in cortical and striatal astrocytes. To examine the impact of repeated cocaine administration on this effect, we then examined the impact of PACAP treatment on ^{14}C-cystine uptake in ex vivo nucleus accumbens tissue punches obtained from rats withdrawn for at least three weeks from daily saline or cocaine self-administration. Interestingly, PACAP-induced enhancement of ^{14}C-cystine uptake in nucleus accumbens tissue punches was not observed in tissue punches obtained from rats withdrawn from repeated cocaine. To determine the behavioral significance of these observations, we microinjected PACAP into the nucleus accumbens and found that this significantly reduced cocaine-primed reinstatement. Collectively, these data are consistent with the possibility that reduced PACAP signaling, possibly as a result of corticostriatal hypofunction, lead to altered glutamate release and clearance by astrocytes in a manner that influences the magnitude of cocaine-primed reinstatement.

Disclosures: L. Kong: None. **A. Madayag:** None. **J.M. Resch:** None. **S. Choi:** None. **J.R. Mantsch:** None. **D.A. Baker:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.09/NN13

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA05312

Title: Chronic non-contingent cocaine alters behavioral measures of urgency in a frustrative reward-omission test

Authors: *A. BARKER, G. V. REBEC;
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Abstract: BACKGROUND: Urgency is a personality sub-trait of impulsivity that serves as a stronger predictor for substance abuse over other forms of impulsivity such as disinhibition or sensation-seeking. It is unknown whether high urgency individuals are intrinsically more susceptible to substance abuse, or if substance abuse itself exacerbates urgency the way psychostimulants are known to enhance other forms of impulsivity. A second unknown is the role of the dopaminergic system in altering the expression of urgency. In this study, rats, previously exposed to cocaine or vehicle, were assessed for urgency in a frustrative reward-omission test. Some rats were treated instead with RTI-113, a selective dopamine transport (DAT) inhibitor. Methods: Adult, male rats received cocaine (ip) in linearly escalating doses beginning at either 10 mg/kg or 20 mg/kg. By the tenth day the doses were twice the initial starting dose, ending at 20 mg/kg or 40 mg/kg. RTI-113 was given (ip) once daily at a constant dose of 5.0 mg/kg, and control subjects received an equal volume of saline on the same schedule. The animals were returned to their home cages following each injection. After the final day of injections, subjects remained in their home cages for 14 days of abstinence prior to beginning of training. Animals were trained on a frustrative reward-omission test (32 trials/day) composed of a classical and operant conditioning portion. A cue predicted receipt of a food-pellet followed by presentation of active and inactive levers for 45 s during which time rats earned as many rewards as possible on an FR10 schedule. On the 4 data collection days there was a 33% chance of free pellet omission, and each day of data was separated by 3 days of extinction where no omission occurred. RESULTS: All treatments showed an urgency effect within groups, validating the test. Cocaine produced a dose-dependent alteration of behavior on omission trials. The high dose cocaine group showed a shorter latency to lever-press and a greater rate of lever-pressing. Neither dose of cocaine resulted in a greater number of presses, however. Interestingly, the RTI-113 group showed a greater number of lever presses on omission trials over all other groups, but did not show any effect on rate or latency to press. CONCLUSION: Cocaine augments responding in a frustrative reward-omission task, a measure of urgency, but our RTI-113 data suggest that the effect of cocaine may not involve the dopaminergic system. Thus, although dopamine may play a role in urgency, other transmitter systems are likely to modulate this response.

Disclosures: A. Barker: None. **G.V. Rebec:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.10/NN14

Topic: C.18. Drugs of Abuse and Addiction

Support: DA12136

RR-03037

Title: Intracellular mechanisms associated with cocaine induced Conditioned Place Preference (CPP)

Authors: *S. K. NYGARD[1,2], A. KLAMBATSEN[2], R. HAZIM[2], B. BALOUCH[1], S. N. CHOWDHURY[1], V. QUINONES-JENAB[1,2], S. JENAB[1,2];
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Abstract: The development and maintenance of cocaine addiction and relapse after abstinence have been shown to depend heavily on learned associations between environmental stimuli and the rewarding properties of the drug. However, not much is known about the molecular mechanisms that lead to the formation of these environmentally cued drug associations. The conditioned place preference (CPP) paradigm exploits the neural circuitry underlying the reward, learning, and memory processes of the brain, allowing us to explore the maladaptive neural and molecular changes that occur during the formation of these associations. The present study investigated the molecular mechanisms underlying cocaine-induced CPP in response to two doses of cocaine (5mg/kg, i.p. or 20mg/kg, i.p.). In addition, we attempted to block the acquisition of cocaine CPP using the NMDA receptor antagonist MK-801. We found that CPP behavior was expressed only in response to the higher dose of cocaine and was associated with an increase in Nucleus accumbens (NAc) ERK phosphorylation (pERK) and Caudate Putamen (CPu) deltaFosB protein levels during CPP testing. Pretreatment of MK-801 (0.025 mg/kg. i.p.)30 minutes prior to cocaine administration during conditioning, blocked the acquisition and expression of cocaine CPP and reduced NAc pERK and CPu deltaFosB levels. This study provides novel evidence for the role of the NMDA receptor and ERK signaling in the expression of cocaine induced CPP.

Disclosures: S.K. Nygard: None. **A. Klambatsen:** None. **R. Hazim:** None. **B. Balouch:** None. **S.N. Chowdhury:** None. **V. Quinones-Jenab:** None. **S. Jenab:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.11/NN15

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA015036

NIH Grant MH51290

Title: The role of N-methyl-D-aspartate receptor co-agonists in cocaine-induced conditioned place preference and locomotor sensitization: Implications for comorbid schizophrenia and substance abuse

Authors: *M. D. PUHL, A. J. BECHTHOLT, J. T. COYLE;
Psychiatry, Harvard Med. School, McLean Hosp, Belmont, MA

Abstract: Schizophrenia is associated with a high susceptibility to substance abuse. Recent research suggests that dysregulation of *N*-methyl-D-aspartate receptor (NMDAR) function may play a role in the pathopyhsiology of both schizophrenia and drug addiction. NMDARs play a critical role in the neuroplasticity that mediates adaptive changes to abused drugs. In addition, hypofunction of NMDARs has been implicated in schizophrenia. This hypofunction may be due to decreased availability of D-serine, an NMDAR co-agonist. Our laboratory has developed two transgenic mouse lines to test the validity of this hypothesis. The first line is a constitutive knockout of the synthetic enzyme that produces D-serine, serine racemase (SR). Null mutants (SR-/-) exhibit NMDAR *hypo*function. The second line is a constitutive knockdown of glycine transporter 1 (GlyT1), which regulates synaptic glycine, another NMDAR co-agonist. Heterozygous mutants (GlyT1+/-) exhibit NMDAR *hyper*function. Previously, we characterized the behavior of these lines in a cocaine-induced (20 mg/kg) conditioned place preference (CPP) and locomotor sensitization paradigm. Briefly, GlyT1+/- mice displayed hastened extinction of cocaine-induced CPP, accompanied by robust cocaine-induced reinstatement, as well as enhanced locomotor sensitization to cocaine. Interestingly, SR-/- mice appeared to immediately forget the learned preference, did not exhibit cocaine-induced reinstatement, and displayed attenuated locomotor sensitization to cocaine. In the current study, both GlyT1+/- and SR-/- mice were tested in the cocaine-induced CPP paradigm used previously, however, GlyT1+/- mice were either treated chronically (17 days) with gavestinel (10 mg/kg on Day 1, 5 mg/kg on Days 2-17), a GMS antagonist, or vehicle, and SR-/- mice were either treated chronically (17 days) with D-serine (300 mg/kg on Day 1, 150 mg/kg on Days 2-17) or vehicle, with the hypotheses that these treatments would normalize the behaviors of GlyT1+/- and SR-/- mice, respectively. Gavestinel caused the GlyT1+/- mice to immediately forget the learned preference and prevented cocaine-induced reinstatement, but had no effect on locomotor sensitization to cocaine. D-serine appeared to have no effect on extinction or reinstatement of cocaine-induced CPP, but completely reversed the deficit in locomotor sensitization to cocaine. These results further elucidate the role of NMDAR co-agonists in cocaine-induced CPP and locomotor sensitization and may shed light on the neural mechanisms underlying co-morbid schizophrenia and substance abuse. This research was supported by DA015036 and MH51290.

Disclosures: M.D. Puhl: None. **J.T. Coyle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties.. F. Consulting Fees (e.g., advisory boards); Abbott, Jansen Pharmaceutical, Puretech, En Vivo. **A.J. Bechtholt:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.12/NN16

Topic: C.18. Drugs of Abuse and Addiction

Support: HMRI Grant

Title: Activity-regulated cytoskeletal protein expression is decreased in the dorsomedial striatum of relapse vulnerable animals: Association with miR-221 and miR-431 expression

Authors: *R. K. QUINN, A. L. BROWN, E. M. LEVI, B. J. GOLDIE, D. W. SMITH, M. J. CAIRNS, C. V. DAYAS;
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Abstract: Compulsive drug-taking has been linked with impairments in synaptic plasticity and dysregulated microRNA (miRNA) expression in the ventral and dorsal striatum respectively. We found that animals characterized as ‘relapse’-vulnerable exhibited deficits in gene expression for synaptic plasticity associated-proteins in the dorsal striatum (DS), most notably, activity-regulated cytoskeletal protein (ARC) and dopamine receptors 1 (Drd1a) and 2 (Drd2). Given the important role of miRNA in negatively regulating mRNA expression and fine tuning synaptic plasticity, we examined the possible contribution of miRNA to the specific changes in mRNA we observed in DS subregions of ‘relapse’-vulnerable animals.
Animals were trained to self-administer cocaine, and subsequently assessed for motivation to take drug, ability to refrain from drug seeking and propensity to relapse to drug seeking. These indices were used to phenotype animals in ‘relapse’-vulnerable or resistant groups animals. Within the DS, miRNA expression profiles were analysed for both ‘relapse’-vulnerable and resilient animals using microarray analysis. Agilent GeneSpring software was used to identify differentially expressed miRNA, with changes confirmed using qPCR Luciferase assays were

used to confirm miRNA regulation of synaptic plasticity genes in vitro.
ARC mRNA expression and protein levels were significantly reduced in the DMS but not DLS of vulnerable animals. Of note two microRNA predicted to regulate, miR-431 and miR-221, were significantly increased in both the DMS and DLS of addiction vulnerable animals. miR-221 has previously been shown to regulate ARC expression in vitro. To determine if miR-431 regulates ARC we used luciferase assays and found that antisense miR-431 increased luciferase expression, indicating miR-431 regulates ARC expression in vitro. Interestingly, we also found that the expression of miR-212 previously linked with compulsive cocaine-seeking, was decreased in the DMS but increased in the DLS of vulnerable rats..
These results support the accumulating evidence that dysregulated miRNA expression can lead to addiction/relapse vulnerability. Specifically our data suggest that negative regulation of ARC by miR-221/mir-431 may be an underlying neurobiological mechanism contributing to the long lasting drug relapse risk. Furthermore, the opposing pattern of miR-212 expression in the DMS and DLS is consistent with distinct roles for these subregions in regulating addiction processes. Future studies are planned to characterize the pattern of miRNA expression changes in DMS and DLS across the addiction cycle.

Disclosures: R.K. Quinn: None. **A.L. Brown:** None. **E.M. Levi:** None. **B.J. Goldie:** None. **D.W. Smith:** None. **M.J. Cairns:** None. **C.V. Dayas:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.13/NN17

Topic: C.18. Drugs of Abuse and Addiction

Support: Commonwealth of Pennsylvania CURE Addiction Center of Excellence

NIH/NIDA T32DA028874

NIH/NIDA P5012756

VA VISN 4 MIRECC

Title: How does the brain “put on the brakes” in addiction? Modeling inhibition of a pre-potent response to affect-positive stimuli

Authors: *A. R. CHILDRESS[1], Y. LI[1], M. GOLDMAN[1], J. SUH[1,2], Z. SINGER[1], R. EHRMAN[1,3], T. FRANKLIN[1], D. LANGLEBEN[1,4], K. YOUNG[1], R. WETHERILL[1], M.

GAWRYSIAK[4], C. O'BRIEN[1];
[1]Dept Psychiat, Univ. PENN Perelman Sch. Med., Philadelphia, PA; [2]Psychiatry, [3]Behavioral Healthy, [4]Behavioral Hlth., Philadelphia Dept. of Veteran's Affairs Med. Ctr., Philadelphia, PA

Abstract: Aims: Some individuals are able to manage the pull of rewards – e.g., food, sex, or drugs of abuse – while others struggle. This struggle is prominent in the addictions: addicted individuals fail to inhibit their "approach response" to rewarding drugs of abuse, despite the clear negative consequences (e.g., loss of family, friends, jobs, health) of this pursuit. Learning how the brain succeeds, or fails, to "put on the brakes" in response to positive stimuli – even in the presence of a negative danger signal – is critical to developing therapeutics to improve inhibitory function in addictions. Toward this goal, we have developed a novel Go-NoGo task that uses valenced (positive or negative) pictures as Go and NoGo stimuli. We are using this novel task to reveal the brain regions involved in "putting on the brakes" to danger signals occurring during a stream of positive stimuli.
Methods: Fast event-related BOLD fMRI at 3T (SPM8) was used to measure brain responses in a cohort of 57 addicted (cocaine, marijuana or prescription opioids; ongoing) adults during performance of a novel Affect Congruent Go-NoGo task with pleasant images (e.g., baby animals) that encouraged approach as Go stimuli (87.5% of trials), and dangerous images that discouraged approach (e.g., scorpions) as NoGo stimuli (12.5% of trials). Pre-planned contrasts compared the brain response during trials for successful (STOPS) vs. failed (ERROR) inhibition.
Results: Successful inhibition trials (STOPS), as compared with failed inhibition trials (ERRORS), were associated with activation of circuitry common to "motor only" Go-NoGo tasks (e.g., d. cingulate, d.l. prefrontal cortex, and d. striatum/putamen; peak $t = 10.16$, $p < 0.0000$ FWE). Brain regions important in the processing of motivational, valenced stimuli (v. pallidum / d. amygdala, and the caudal orbitofrontal cortex) were also activated during successful inhibition in the novel task.
Conclusion: Successful inhibition in the novel Affect-congruent Go-NoGo was linked to classic "brain brakes" circuitry (d. cingulate, d.l. prefrontal cortex, and d. striatum). Successful inhibition in this novel task was also reflected in the activation of motivational circuitry – a feature that is not captured by standard Go-NoGo tasks with unvalenced, arbitrary stimuli (e.g., letters, numbers or shapes). The novel task may thus provide a closer model to the clinical deficits in addiction – e.g., failure to inhibit approach to drug reward, despite potential dangers. In ongoing work, we are testing the ability of this new task to identify individuals at risk for relapse, and to screen candidate medications for their ability to boost the "brain brakes".

Disclosures: A.R. Childress: None. **Y. Li:** None. **M. Goldman:** None. **J. Suh:** None. **Z. Singer:** None. **R. Ehrman:** None. **T. Franklin:** None. **D. Langleben:** None. **K. Young:** None. **R. Wetherill:** None. **M. Gawrysiak:** None. **C. O'Brien:** None.

Poster

546. Cocaine: Neural Mechanisms of Addiction IV

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.14/NN18

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA21261

Title: An escalating dose regimen of cocaine leads to site-specific changes in neuropeptide Y immunoreactivity in the rat brain

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Abstract: Emerging evidence suggests that neuropeptide Y (NPY) acts on neural substrates that underlie drug use and dependence. Psychostimulants and other drugs of abuse regulate synthesis and release of NPY in the brain and increasing evidence suggests that repeated exposure to these compounds result in neural adaptations that persist through the first few months of abstinence from chronic cocaine exposure. Our laboratory has been investigating the effect of acute manipulation of NPY in the brain on the reinstatement of cocaine seeking behavior during periods of abstinence and we have found that increasing NPY attenuates reinstatement of cocaine-mediated behaviors. The current study was designed to determine if our model of cocaine exposure, an escalating dose regimen in rats, alters NPY after short and long periods of abstinence. To this end, we used immunohistochemistry, which has better anatomical resolution than the techniques used in earlier reports. Male Long-Evans (hooded) rats were given daily injections of cocaine (COC) using an escalating cocaine dose regimen (5-30 mg/kg IP), or vehicle (VEH) for 21 days. Either 1 or 22 days after the final cocaine injection, brains were harvested, cut into 40 µm sections, and every tenth section was processed for NPY-immunoreactivity A significant interaction between cocaine treatment and length of abstinence was found in most forebrain areas. This interaction was due to (1) a decrease in NPY density in the COC-22 d group, with the greatest effect occurring in subregions of the medial prefrontal cortex (i.e. cingulate cortex), and (2) an increase in NPY density the COC-1 d group in a limited number of areas (i.e. infralimbic cortex and prelimbic cortex). The possibility of an effect of age difference on NPY density between rats killed 1 d or 22 d after the last injection was controlled by the experimental design and also evaluated; no effect of age on the interaction between cocaine treatment and length of abstinence was found in preliminary analysis. These results add to a growing body of literature showing that repeated cocaine exposure with abstinence decreases multiple cellular and molecular measures of NPY in forebrain structures and supports the idea that the behavioral sequelae, consequent to cocaine dependence and manifest during abstinences, may be mediated by changes in NPY activity.

Disclosures: M. Suarez: None. **C.P. King:** None. **D. Daniels:** None. **A.C. Thompson:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.15/OO1

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA025617 (DB)

NIH Grant DA035088 (DB & SC)

NIH Grant DK074734 (SC)

Title: Mutating Slc7A11 to create a system xc- knockout rat

Authors: *S. CHOI[1], A. GEURTS[2], J. RESCH[1], N. RADDATZ[1], L. KONG[1], J. CLELAND[1], J. MANTSCH[1], D. BAKER[1];
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Abstract: Glutamate is often described as the primary excitatory neurotransmitter in the brain yet we are only now unmasking how the many components of this complex network of receptors, membrane-expressed enzymes, transporters, and release mechanisms function in an integrated manner to regulate the neurotransmitter actions of this amino acid. System x_c^-, which is highly expressed by astrocytes, is an example of a poorly understood source of glutamate release that functions as a cystine-glutamate antiporter that couples the uptake of one molecule of cystine to the release of one molecule of glutamate. As a result, system x_c^- is at the interface between excitatory signaling and oxidative stress making it an intriguing target in attempts to understand the pathological states of the central nervous system, especially as it relates to astrocytic control of the primary excitatory neurotransmitter in the brain. To better understand the contribution of system x_c^- to multiple aspects of brain functioning, we developed transgenic rats by mutating the Slc7A11 gene using Zinc-Finger Nucleases (ZFN). Genotyping data revealed that efforts to mutate Slc7A11 were successful in two lines of Sprague Dawley rats. The first line (M1) contains a 39 base pair deletion in exon 2 leading to a frameshift and anticipated truncation of the xCT protein. The second line (M2) has a deletion of 13 amino acids corresponding to the majority of the 3rd transmembrane domain. We have confirmed that the M1 mutation results in a complete elimination of system x_c^- activity by demonstrating a lack of system x_c^- dependent uptake of ^{14}C-cystine in striatal tissue punches. We will use these rats to determine the role of

system x_c^- in multiple behaviors used to model aspects of drug addiction and schizophrenia.
DA025617 (DB), DA035088 (DB and SC), DK074734 (SC)

Disclosures: S. Choi: None. **A. Geurts:** None. **J. Resch:** None. **N. Raddatz:** None. **L. Kong:** None. **J. Cleland:** None. **J. Mantsch:** None. **D. Baker:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.16/OO2

Topic: C.18. Drugs of Abuse and Addiction

Support: DA029565

DA028020

DA023206

DA024570

DA031551

Title: Contingent cocaine exposure regulates inhibitory synaptic transmission in the nucleus accumbens

Authors: *M. OTAKA[1], M. ISHIKAWA[1], B. R. LEE[3], L. LIU[1], P. A. NEUMANN[1], Y. H. HUANG[2], O. M. SCHLÜTER[4], Y. DONG[1];
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Abstract: Addiction is known as a relapsing chronic psychiatric disorder. The high possibility of relapse significantly interrupts the treatment of addiction. It has been well characterized that drug addiction causes dynamical cellular adaptations in medium spiny neurons (MSNs) within the nucleus accumbens shell (NAc). The neuronal output NAc MSNs is mainly determined by the integration of membrane excitability and excitatory/inhibitory synaptic inputs. Cocaine-induced alterations at excitatory synapses and membrane excitability have been extensively examined. However, the overall functional output of NAc MSNs following cocaine exposure is still poorly defined because little is known whether inhibitory synaptic input to MSNs is also affected by cocaine. Our results showed alterations at inhibitory synapses in MSNs at NAc shell following cocaine self-administration in rats. Specifically, the amplitude of miniature IPSCs (mIPSCs) was

decreased after 3 weeks WD from 5 d cocaine self-administration. Upon re-exposure to cocaine after 3 weeks WD, the frequency of mIPSCs became significantly higher. Furthermore, the reversal potential of IPSCs, which was not significantly altered during WD, became more hyperpolarized upon cocaine re-exposure. Moreover, the relative weight of excitatory and inhibitory inputs to NAc MSNs was significantly decreased after 1 d cocaine WD, increased after 3 weeks WD, and returned to the basal level upon cocaine re-exposure after 3 weeks WD. Taken together with previous results showing cocaine-induced adaptations at excitatory synapses and intrinsic membrane excitability of NAc MSNs, our present results may provide overall picture of the functional state of NAc MSNs following cocaine exposure and possible therapeutic approaches for cocaine addiction.

Disclosures: M. Otaka: None. **M. Ishikawa:** None. **B.R. Lee:** None. **L. Liu:** None. **P.A. Neumann:** None. **Y.H. Huang:** None. **O.M. Schlüter:** None. **Y. Dong:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.17/OO3

Topic: C.18. Drugs of Abuse and Addiction

Support: CIHR (grant 89904)

NSERC

NARSAD Young Investigator Award

Title: An adaptive role for TNFα mediated plasticity in response to cocaine

Authors: *G. M. LEWITUS, S. KONEFAL, D. STELLWAGEN;
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Abstract: Repeated cocaine administration produces enduring molecular, cellular and behavioral plasticity associated with changes in striatal glutamatergic synapses. The mechanism underlying this type of plasticity, however, is not well understood. In addition to its role in the inflammatory response, the cytokine TNFα is elevated by drugs of abuse and has been shown to regulate AMPA receptor (AMPAR) trafficking. On hippocampal and cortical pyramidal cells, exogenous application of TNFα leads to a dramatic and rapid exocytosis of AMPA receptors. In contrast, medium spiny neurons (MSNs) in the striatum respond to TNFα by endocytosing AMPA receptors. The aim of the present study is to investigate the role of TNFα in the striatal plasticity

induced by cocaine. We use patch-clamp recordings to investigate the contribution of TNFα to the regulation of AMPAR trafficking on MSNs in the nucleus accumbens and its relevance to behavioral sensitization to cocaine. We find that the TNFα -/- mice display an increased locomotor response as compared to wildtype mice following a behavioral sensitization protocol. Further, we find that pharmacologically blocking TNFα signaling during sensitization, but not during the withdrawal period is sufficient to increase behavioral responses. Moreover, the increased behavioral sensitization in TNFα -/- is associated with altered glutamatergic synaptic strength in the nucleus accumbens. These results suggest that TNFα-mediated striatal plasticity is a homeostatic response to cocaine administration.

Disclosures: G.M. Lewitus: None. **S. Konefal:** None. **D. Stellwagen:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.18/OO4

Topic: C.18. Drugs of Abuse and Addiction

Support: GAANN Fellowship

NIH MH081004

NIH DA025922

NINDS T32 training grant NS045540

NIDA F32DA031520

Title: Role of HDAC3 in modulating acquisition/consolidation and extinction of cocaine-induced conditioned place preference

Authors: *A. WHITE;
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Abstract: Histone acetylation is a key mechanism of gene expression control involved in several aspects of drug-seeking behavior. Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which facilitate and repress transcription, in general. In this study, we specifically examined the role of HDAC3, the most prominently expressed HDAC in the brain, in the acquisition/consolidation and extinction of cocaine-induced conditioned place preference (CPP). We have previously demonstrated that HDAC3 is a critical

negative regulator of long-term memory formation. As cocaine-induced CPP is an associative memory task, we predicted that genetic deletion and/or pharmacologic inhibition of HDAC3 would facilitate acquisition/consolidation and also facilitate extinction. Indeed, Hdac3-FLOX mice with a focal deletion in the nucleus accumbens showed significantly enhanced CPP, which correlated with increased histone acetylation at H4K8 and increased expression of immediate early genes c-Fos and Nr4a2. Chromatin immunoprecipitation demonstrated that increased gene expression of these genes is associated with the loss of HDAC3 at their promoters. These results show that HDAC3 negatively regulates cocaine-induced CPP. To examine extinction, we turned to a pharmacological approach in which an HDAC3 specific inhibitor was used to block HDAC3 activity during the consolidation phase of extinction memory formation. HDAC3 inhibition significantly enhanced extinction of cocaine-induced CPP. Similar to our previously published memory findings, HDAC3 inhibition not only facilitated extinction, but generated a form of extinction that is persistent and that blocks reinstatement. Additionally, HDAC3 inhibition during extinction promoted a distinct pattern of histone acetylation linked to gene expression within the infralimbic cortex, hippocampus, and nucleus accumbens. Together, these results demonstrate that HDAC3 is a key enzyme involved in the acquisition/consolidation and extinction of drug-seeking behavior.

Disclosures: A. White: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Religen Corporation.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.19/OO5

Topic: C.18. Drugs of Abuse and Addiction

Title: Manipulation of moesin protein levels in the nucleus accumbens core regulates cocaine-induced locomotor activity

Authors: W. KIM[1], J. JANG[1], B. CHO[1], J. LEE[1], W. CAI[1], S. JEON[2], *J.-H. KIM[1];
[1]Dept of Physiol., Yonsei Univ. Coll Med., Seoul, Korea, Republic of; [2]Res. Inst. of Biotech., Dongguk Univ., Seoul, Korea, Republic of

Abstract: The ezrin-radixin-moesin (ERM) proteins have been implicated not only in cell-shape determination but also in cellular signaling pathway. Previously, we showed that cocaine

decreases the phosphoryaltion levels of ERM in the NAcc in a time- and dose-dependent manner. However, it has not been determined yet what functional role ERM proteins play in the nucleus accumbens (NAcc) in relation with drugs of abuse. Here we microinjected lenti viral vectors, which contains either wild-type or mutant moesin gene, into the NAcc core and measured locomotor activity following either saline or cocaine (15 mg/kg, IP) injections. Interestingly, over-expression of wild-type mosein in this site inhibited cocaine-induced hyper-locomotor activity, while mutant gene further enhances it, compared vehicle microinjection groups. In saline treated rats, both viral vectors produced locomotor activity not different from vehicle microinjection groups. These results first time indicate that moesin-mediated signaling pathways in the NAcc core contribute to cocaine-induced locomotor activity. Considering the fact that there is very little known about the functional role of ERM proteins in the brain in the literature, our present findings will open a new venue especially bringing ERM proteins to our attention in terms of psychomotor stimulants-related signaling pathways.

Disclosures: W. Kim: None. **B. Cho:** None. **J. Lee:** None. **W. Cai:** None. **S. Jeon:** None. **J. Kim:** None. **J. Jang:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.20/OO6

Topic: C.18. Drugs of Abuse and Addiction

Support: Hatos Foundation

NIH Grant DA005010

Title: Beta-arrestin 1 dependent regulation of cocaine self-administration in mice

Authors: *N. MITTAL[1], Z. ABDULLA[2], A. M. JAMES[3], D. JENTSCH[3], C. CRAWFORD[2], C. EVANS[3], W. WALWYN[3];
[1]Univ. of Texas At Austin, Austin, TX; [2]California State Univ. San Bernardino, San Bernardino, CA; [3]Univ. of California Los Angeles, Los Angeles, CA

Abstract: AMPARs (2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid receptors) rely on the 3-dimensional structure of the postsynaptic density to be trafficked into and removed from the synapse as necessary. This is mediated by the rapid rearrangement of actin filaments, which is orchestrated by a series of proteins, including the actin severing protein, cofilin, Lim domain kinase (LIMK), the upstream kinase, and Slingshot, a phosphatase. These proteins are in

turn associated with one of the two non-visual arrestins, thereby providing spatiotemporal regulation of actin turnover. These protein interactions predict that mice lacking one of the arrestin isoforms, β-arrestin 1, may demonstrate altered AMPAR function. As changes in AMPAR subunit composition and function are a well-recognized hallmark of single and repeated exposure to psychostimulants such as cocaine, we examined whether mice lacking B-arrestin1 show an altered profile of cocaine self-administration and of AMPAR function. We first defined the profile of intravenous self-administration of cocaine in β-arrestin 1 knockout (βarr1-/-) and wildtype (WT, βarr1+/+) mice. Mice were trained to self-administer cocaine (1 mg/kg/infusion) on schedules of reinforcement that progressively advanced: fixed ratio (FR) 1, FR2, and FR5. Sessions were conducted on sequential days and each lasted 2 hours. Relative to their WT littermates, βarr1-/- mice earned fewer cocaine infusions during the early stages of acquisition (at FR1; p=0.016), but not thereafter (at FR2 or FR5). After stable responding at FR5 was achieved, 5 days of extinction followed, in which the mice were placed in the operant boxes but no drug delivered when the active lever was pressed. During early extinction learning WT mice showed a faster decline in the number of lever presses as compared to βarr1-/- mice (p=0.015). Thereafter, both genotypes showed equivalent cue-induced reinstatement behaviors. At the end of either the acquisition or reinstatement phases, mice were removed from the study to assess the AMPA/NMDA (A/N) ratio from evoked excitatory post-synaptic currents (eEPSCs) in medium spiny neurons in the nucleus accumbens shell. We found that the A/N ratio from naive WT mice was lower than that of naïve βarr1-/- mice (p=0.047). Acquisition and reinstatement of cocaine self-administration was associated with a progressive increase in the A/N ratio in WT mice. In contrast, the A/N ratio from βarr1-/- was not altered at any stage. These data suggest that β-arrestin 1 plays an important role in the acquisition and extinction of cocaine self-administration, and in the adaptations of AMPAR function that may underlie these behaviors.

Disclosures: N. Mittal: None. **Z. Abdulla:** None. **A.M. James:** None. **D. Jentsch:** None. **C. Crawford:** None. **C. Evans:** None. **W. Walwyn:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.21/OO7

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA005130

NIH Grant DA004600

NIH Grant HL096571

Title: Expression of the HIV-1 glycoprotein, gp120, increases ROS production and glutamate NMDA receptor density in the dorsal striatum following repeated systemic cocaine administration

Authors: *D. A. LANE, M.-K. LYNCH, G. WANG, C. IADECOLA, V. M. PICKEL;
Brain and Mind Res. Inst., Weill Med. Col. of Cornell Univ., NEW YORK, NY

Abstract: Human immunodeficiency virus (HIV) infection is associated with increased susceptibility for drug addiction, which may be indicative of convergent intracellular signaling in targeted brain regions. The production of reactive oxygen species (ROS) in response to glutamate activation of NMDA receptors plays an important role in both neurodegenerative and addictive diseases suggesting that this pathway may be a key mechanism involved in this convergent signaling. We tested this hypothesis using a cocaine model and examining changes in the mouse dorsal striatum, which is one of the regions most notably involved in HIV associated motor dysfunctions and in the emergence of drug-seeking motor habits that are dependent on both dopamine and NMDA receptor mediated glutamatergic transmission. Cocaine (15 mg/kg/day) was administered for 14 consecutive days in wild-type (WT) and transgenic male mice that over express GFAP-gp120 (gp120+), followed by behavioral testing. Isolated striatal cells from each treatment group were processed for ROS detection using dihydroethidine fluorescence imaging. Tissue sections through the striatum of the same experimental groups were processed for dual immunogold labeling of NMDA NR1 subunit and peroxidase identification of the dopamine D1 receptor, a receptor that is often co-mobilized with NR1 in striatal neurons. Both gp120+ and WT control mice showed sensitization to the stimulant effects of cocaine (3 fold increase in distance traveled) and conditioned place preference to cocaine-paired environments as compared to saline controls. Whereas, chronic cocaine attenuated the production of NMDA-elicited (100 μM) ROS in WT mice, it significantly increased ROS in gp120+ mice (1.04 vs. 1.20 ± 0.03 standard ROS units, $p < 0.01$). Further, the change ROS production was associated with increased NMDA receptor density, both at the plasma membrane and in the cytoplasm, of dopamine D1-receptor containing dendrites within the dorsal striatum of gp120+ mice. Taken together, the data suggest that the presence of gp120 augments cocaine-induced activation of dopamine receptive striatal neurons, which can increase cocaine craving and seeking through mechanisms that involve NMDA receptor-mediated ROS production.

Disclosures: D.A. Lane: None. **M. Lynch:** None. **G. Wang:** None. **C. Iadecola:** None. **V.M. Pickel:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.22/OO8

Topic: C.18. Drugs of Abuse and Addiction

Support: Scientific Research Grant from the MEXT of Japan

Title: Cellular mechanisms of enhanced cocaine self-administration in mhc class I deficient mice

Authors: *G. MURAKAMI, H. MENG, M. EDAMURA, D. NAKAHARA;
Dept. of Psychology, Hmamatsu Univ. Sch., Shizuoka, Japan

Abstract: Brain was thought to be an immune privilege region, however, it has been recently discovered that various immune proteins have important roles even in the brain. Particularly major histocompatibility complex class I (MHCI) is critically involved in the organization of neural circuits in development and learning and memory in adults. Given that cocaine abuse activates the immune system in peripheral organs, it is reasonable to hypothesize that cocaine modulates the expression of MHCI and changes the synaptic plasticity in brain regions contributing to cocaine addiction. Our previous experiment showed that mice with functionally deficient MHCI exhibited enhanced locomotor sensitization induced by repeated cocaine injection in comparison with their wild type. In self-administration paradigm, functional MHCI deficient mice showed much more addictive behavior such as enhanced locomotor activity and nose-poking than wild type mice after 10 days of cocaine administration and 10 days of abstinence followed by a 3-day reinstatement. This result showed a critical involvement of MHCI in cocaine addiction.
To assess whether cocaine modified expression of MHCI in the brain, we compared the mRNAs of H2D and H2K by qPCR in brain regions such as the ventral tegmental area (VTA), accumbens (Acb), prefrontal cortex (PFC), amygdala (Amy) and hippocampus (Hip). Cocaine decreased H2D expression only in the VTA after the injection for 7 days followed by 10 days of abstinence. The decrease of H2D expression in the VTA was also observed in the mice exposed to self-administration paradigm. We also confirmed that H2D was expressed in dopaminergic neurons in the VTA by immunohistochemistry with membrane-nonpermeabilization method using several antibodys. Now we are investigating the role of MHCI in the cocaine addiction at a level of electrophysiological properties of dopaminergic neurons in the VTA. To confirm the reduction of H2D particularly in the VTA contributing to the process of cocaine addiction, we are also trying to manipulate the expression of H2D in the VTA using adeno-associated virus vector.

Disclosures: G. Murakami: None. **H. Meng:** None. **M. Edamura:** None. **D. Nakahara:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.23/OO9

Topic: C.18. Drugs of Abuse and Addiction

Support: Wellcome Trust

Dana Foundation

McKnight Foundation

Title: Cocaine exposure alters pathway-specific synaptic connectivity in the Nucleus Accumbens

Authors: *A. MACASKILL, J. M. CASSEL, A. G. CARTER;
New York Univ., New York, NY

Abstract: Alterations in the synaptic properties of medium spiny neurons (MSNs) in the Nucleus Accumbens (NAc) are thought to play a key role in the transition to addiction. MSNs in the NAc are segregated into the direct and indirect pathways, which have opposing effects on reward-related behaviors. Drug induced changes in synaptic properties are thought to change the balance of the two pathways, resulting in altered basal ganglia output. However, MSNs process diverse long-range excitatory synaptic inputs from multiple brain regions, each with distinct properties and functions. Here we use a combination of electrophysiology, two-photon microscopy and optogenetics to study the impact of drug exposure on NAc circuitry. We find alterations in the synaptic organization of the NAc circuit at the subcellular level of spines and dendrites. These changes are input specific, with inputs from the ventral hippocampus and basal amygdala each displaying unique functional alterations. Thus, drug induced alterations in NAc circuitry are not homogenous, but depend upon the presynaptic origin of the synaptic contact.

Disclosures: A. Macaskill: None. **J.M. Cassel:** None. **A.G. Carter:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.24/OO10

Topic: C.18. Drugs of Abuse and Addiction

Support: DA011806

MH061933

Title: Sex differences in girk signaling in layer 5/6 pyramidal neurons of the mouse prefrontal cortex

Authors: *E. MARRON, M. HEARING, K. WICKMAN;
Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Glutamatergic pyramidal neurons in the deepest layers (Layers 5/6) of the medial prefrontal cortex (mPFC) are the primary output neurons of the mPFC and as such, play a key role in modulating neurotransmission in the mesocorticolimbic system. Studies in both humans and rodents have demonstrated sexually-dimorphic behavioral responses to cocaine and cocaine-related cues including increased behavioral activation, place preference, and reinstatement of drug-seeking. This sex disparity has been linked to intrinsic differences in inhibitory G protein-coupled receptor (GPCR) function in the mesocorticolimbic system, including differential D_2R activation in the mPFC. $GABA_B$ receptor-dependent signaling also plays a key role in regulating mPFC glutamatergic output. The postsynaptic inhibitory influence of $GABA_B$ and D_2R is mediated in many neurons by G protein-gated inwardly-rectifying K^+ (Girk/$K_{IR}3$) channels. Thus, we investigated whether sex differences exist in mPFC Layer 5/6 pyramidal neuron $GABA_BR$-Girk signaling. Somatodendritic currents induced by the $GABA_BR$ agonist baclofen were measured using the whole-cell voltage-clamp technique in Layer 5/6 pyramidal neurons in acutely-isolated coronal slices from adolescent male and female C57BL/6 mice. Baclofen triggered robust outward currents ($I_{Baclofen}$; V_{hold}= -60 mV) in both male and female mice that reversed near E_K (-92 mV) and correlated with decreased input resistance, features consistent with the activation of a K^+ channel. $I_{Baclofen}$ was significantly larger in wild-type male mice compared to age-matched female counterparts. While $I_{Baclofen}$ was markedly reduced in male mice lacking either Girk1 or Girk2 subunits, no significant differences in $I_{Baclofen}$ were seen in female wild-type and *Girk*$^{-/-}$ mice. Importantly, Layer 5/6 pyramidal neurons in wild-type females also displayed a reduction in the current needed to evoke an action potential and enhanced current-evoked action potential firing compared to wild-type males. Taken together, these data suggest that female mice display intrinsically lower Girk-dependent signaling in Layer 5/6 mPFC pyramidal neurons, which promotes enhanced excitability of these neurons. Since increased excitability of Layer 5/6 mPFC pyramidal neuron excitability and neurotransmission are critical for the expression of cocaine-related behavior, intrinsic differences mPFC Girk-related signaling may contribute to differences between males and females in their response to cocaine.

Disclosures: E. Marron: None. **M. Hearing:** None. **K. Wickman:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.25/OO11

Topic: C.18. Drugs of Abuse and Addiction

Title: Antioxidant compounds modulate cocaine-conditioned effects on locomotion

Authors: *J. D. NGUYEN, M. J. FORSTER;
Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: It was hypothesized that an acutely reducing cellular redox state could attenuate redox signals involved in neural plasticity events mediating associations of context with psychostimulant exposure. To test this hypothesis, three compounds with different antioxidant actions were evaluated for their ability to affect the acquisition and expression of context-dependent increases in locomotion produced by a single exposure to cocaine. Cocaine (40 mg/kg) was administered to different groups of Swiss-Webster mice via intraperitoneal injection (i.p.), in either a locomotor activity testing apparatus or the home cage, 2 hours following an activity test under saline. Mice placed in the testing chambers were given 30 minutes to explore freely and locomotion was monitored using a Digiscan photocell apparatus. A conditioned effect of cocaine was inferred by an increase in horizontal activity counts relative to home cage cocaine controls during a test in the same apparatus on the following day. For testing of effects on expression of the conditioned cocaine effect, N-acetylcysteine (25, 50, 100 mg/kg), dimethylthiourea (5, 10, 25, 50 mg/kg), L-ascorbic acid (25-500 mg/kg), or vehicle was administered prior to placement into the activity chambers on the test day. The same compounds were administered prior to the acquisition day in a separate set of studies. N-acetylcysteine (100 mg/kg) and dimethylthiourea (25 and 50 mg/kg) inhibited the expression and the acquisition of cocaine-conditioned locomotion, though L-ascorbic acid (100 mg/kg) increased the acquisition of the conditioned locomotor effect and did not affect its expression. Additionally, L-ascorbic acid was found to facilitate the acute motor stimulant effect of cocaine, whereas N-acetylcysteine and dimethylthiourea did not affect the locomotor response to cocaine. The ability of these compounds to inhibit or exacerbate the conditioned behavior suggests that alteration of redox state may indeed influence neural plasticity dependent alterations in brain mediating addiction and relapse. Therefore, compounds producing alterations in cellular redox state may be viable targets for addiction treatment medications.

Disclosures: J.D. Nguyen: None. **M.J. Forster:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.26/OO12

Topic: F.02. Animal Cognition and Behavior

Title: The effect of acupuncture on the cocaine-induced anhedonia

Authors: *S. IN[1], B. LEE[1], H. HAN[1], H. KIM[2], S. YOON[2], C. YANG[2], S. LEE[3], R. ZHAO[5], S. LIM[1], J. KIM[1], Y. LEE[1], H. LEE[1], T. JUNG[4], S. IN[1];
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Abstract: Objectives: Anhedonia often appears in the psychiatric disorders such as schizphrenia and depression. It is a kind of side effect of antidopaminergic drugs and easily caused by withdrawal from the abused drugs like cocaine or amphetamine that enhances dopaminergic neurotransmission. The purpose of this study was to examine the effects of acupuncture on the anhedonia induced by cocaine and to investigate possible neuronal involvement.
Material & Methods: Male Wister rats were trained to self-administer 4% sucrose solution under a fixed ratio (FR) schedule using 2 h session for 10 days (Day 1-3: FR 1, Day 4-6: FR 3, Day 7-10: FR 10, 0.1ml per infusion) and rats who had established stable response were trained with progressive ratio (PR) schedule. When animals had taken no more sucrose solution for 1 h, the number of infusions was defined as break point. After 10 days of PR training, animals that established stable baseline (variation less than 10% of the average for three consecutive days) on the break point received cocaine 24 h after the establishment of baseline. Cocaine (15 mg/kg) or saline was given total 8 times intraperitoneally with an interval of 90 min with the exception of the first interval of 30 min. Thereafter, animals were re-exposed to the sucrose self-administration 12 h, 36 h, and 60 h after the finish of cocaine injection. Acupuncture was applied at each acupoint immediately before the start of re-exposure to sucrose. GABAA receptor antagonist, bicuculline (1.0 mg/kg), and GABAB receptor antagonist, SCH 50911 (2.0 mg/kg), were given 20 min prior to the acupuncture.
Results: It has been shown that animals that had been exposed to cocaine exhibited decreased sucrose-seeking behavior and that acupuncture at SI5, but not at the control acupoint LI5, significantly increased the sucrose self-administration. And this effect of acupuncture was blocked by the treatments of GABA receptor antagonists (GABAA and GABAB).
Conclusions: The results of this study demonstrate that acupuncture at SI5 may be effective in the treatment of anhedonia caused by exposure to cocaine and that this effect is mediated by, at least in part, GABA receptor system.

Disclosures: S. In: None. **B. Lee:** None. **H. Han:** None. **H. Kim:** None. **S. Yoon:** None. **C. Yang:** None. **S. Lee:** None. **R. Zhao:** None. **S. Lim:** None. **J. Kim:** None. **Y. Lee:** None. **H. Lee:** None. **T. Jung:** None. **S. In:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.27/OO13

Topic: F.03. Motivation and Emotion

Support: NHMRC 633267

Title: Acceleration of habit learning following cocaine sensitization and reversal by N-acetylcysteine

Authors: *L. H. CORBIT[1], B. C. CHIENG[2], B. W. BALLEINE[2];
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Abstract: Drug addiction is defined primarily by behavioural characteristics related to loss of control over drug use. Despite an increasing number of studies examining the effects of stimulant exposure on drug seeking and taking, there has been little systematic research into how these drugs influence normal decision-making.
Repeated exposure to drugs of abuse causes a wide range of long-lasting neural adaptations, however, to date, this work has not established whether these changes are sufficient to lead to executive dysfunction. Of note, several recent reports show that repeated amphetamine exposure, sufficient to induce sensitization, accelerates the loss of volitional control over reward seeking, causing abnormally rapid development of habitual reward seeking.
Chronic drug exposure has been demonstrated to impair glutamate homeostasis by altering the balance between synaptic and extra-synaptic glutamate release and elimination (see Kalivas, 2009). Drugs that restore this balance have been shown to reduce drug-seeking behaviours. For example, treatment with N-acetlycysteine (NAC), prevents behavioural sensitization and reinstatement of cocaine self-administration. These findings raise the intriguing hypothesis that NAC may also prevent the abnormally rapid formation of habits after stimulant exposure and help to restore normal decision-making processes.
The following experiments examined whether pre-training exposure to cocaine accelerates habit learning (defined as loss of sensitivity to outcome devaluation) and whether co-treatment with NAC could prevent the effects of cocaine on subsequent learning. Ex-vivo electrophysiological

experiments examined changes in glutamatergic signaling following cocaine pre-treatment in the dorsomedial (DMS) and dorsolateral (DLS) regions of the dorsal striatum previously shown to underlie goal-directed and habit learning, respectively. We find that cocaine exposure accelerates habit learning and this effect is prevented by co-treatment with NAC suggesting that dyregulation of glutamate homeostasis contributes to accelerated habit learning following drug exposure. Furthermore, spontaneous glutamate release was found to be elevated in the DMS following cocaine exposure suggesting that the rapid shift from goal-directed to habitual control following drug exposure relates to dysregulation of the goal-directed system permitting early dominance by the habit system rather than direct effects on plasticity in structures controlling habit learning.

Disclosures: L.H. Corbit: None. **B.W. Balleine:** None. **B.C. Chieng:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.28/OO14

Topic: D.08. Pain

Support: Texas Norman Hackerman Advanced Research Program (003656-0071-2009)

TxMRC Grant

Title: Local field potentials in the ventral tegmental area correlate with cocaine-induced locomotor activation: Measurements in freely moving rats

Authors: *A. HARRIS[1], A. LI[2], J. E. SIBI[2], S. A. MORRIS-BOBZEAN[2], Y. PENG[2], L. I. PERROTTI*[2];
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Abstract: It is well established that the ventral tegmental area (VTA) is a critical nucleus for the development of drug-related changes in behavior. For example, when administered acutely, the psychostimulant cocaine activates neurons in the VTA (and other regions of the mesocorticolimbic reward pathway) and results in an increase in locomotor behavior. However, our current knowledge of how acute drug-induced changes in the firing rate of local field potentials in reward related circuitry correlate with drug-induced behaviors in real-time are limited. The use of our custom-designed wireless recording module presents a unique opportunity to assess changes in neuronal activity within the VTA of freely moving rats during

cocaine-induced locomotor activation. The overall goal of this study was to test the hypothesis that changes in field potential activity in the VTA would increase with increases in locomotor behaviors after the administration of a single cocaine injection. Adult female rats received a unilateral implant of an electrode in the VTA (5.8 posterior to bregma, 2.0mm lateral to the right, and 8.3 mm from dura at an angle of 10 degrees). Field potentials and locomotor behaviors were simultaneously recorded in freely-moving rats for a 30 minute baseline period; again for 30 minutes following an injection of NaCl (0.9% NaCl at 0.1 ml/kg, i.p.), and a third time for 60 minutes following an injection of cocaine hydrochloride (10mg/kg, i.p.). Field potentials were analyzed by power spectrum in Spike 2 (CED). Different frequency bands (Delta,>4 Hz; Theta, 4-8 Hz; Alpha,8-13 Hz; Beta,>13-30 Hz; and Gamma, 30-100+ Hz) were processed using an ANOVA, followed by posthoc LSD tests. Results showed significant increases in locomotor behaviors after cocaine administration when compared to baseline and saline time points, ($p<.05$). More specifically, we observed significant increases in field potential activity ($p<.05$) at all frequency bands at 30 min and 60 min following cocaine administration. Further analyses revealed substantial increases in delta and theta waves within these field potentials. Our study has provided data to support the use of our custom-designed wireless recording module to further explore the neural activity during drug-induced behavioral activation.

Disclosures: A. Harris: None. **A. Li:** None. **J.E. Sibi:** None. **S.A. Morris-Bobzean:** None. **Y. Peng:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Texas Norman Hackerman Advanced Research Program (003656-0071-2009) and TxMRC Grant. **L.I. Perrotti*:** None.

Poster

546. **Cocaine: Neural Mechanisms of Addiction IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 546.29/OO15

Topic: C.18. Drugs of Abuse and Addiction

Title: Effects of a positive allosteric modulator of the metabotropic glutamate receptor 5 within the Nucleus Accumbens shell during environmental elicited cocaine conditioning

Authors: *K. TORRES, A. MARTÍNEZ-RIVERA, L. GONZÁLEZ, C. S. MALDONADO-VLAAR;
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Abstract: Recent data from our laboratory suggest that the associative learning required for cocaine conditioning is disrupted by mGluR5 antagonist treatment within the NAc. Specifically, we demonstrate that mGluR5 blockade decreased environmental-elicited cocaine conditioning response. This significant reduction was not found in the control or the cocaine-unpaired subjects. Our results imply that mGluR5 could be modulating the memory processes regulated within the NAc essential for the association of environmental cues with cocaine effects. In contrast, previous results report that mGluR5 positive allosteric modulators (PAMs) enhance synaptic plasticity, improve spatial learning and its a beneficial effects in the treatment of cognitive impairment associated with schizophrenia. Moreover, limited numbers of studies have examined the effects of mGluR5 PAMs on cocaine addiction. However, no study to date has investigated the therapeutic potential of other mGluR5 PAM novel compounds on specific aspects of cocaine addiction such as conditioned locomotor response. Therefore, this research seeks to unveil the role that a selective PAM of mGluR5 within NAc shell has on cocaine conditioning. Our hypothesis is that the mGluR5 selective agonist CHPG (RS)-2-chloro-3-hydroxyphenylglycine will promote and enhance the expression of the cocaine conditioned response. Rats were implanted with cannulas within NAc shell, and separate groups were exposed to a multimodal environment within activity chambers that signaled cocaine (paired) or saline (controls, unpaired). Prior to placing the animals in the chambers, rats received systemic injections of saline or cocaine for 10 consecutive sessions. On the day of the conditioned response expression, different groups of rats were microinjected with vehicle or the agonist CHPG into the NAc shell and then they were placed in the activity chambers. Interestingly, results show no change in the expression of the conditioned response in cocaine paired experimentally treated animals. Future studies are needed to further characterize these effects.

Disclosures: K. Torres: None. **A. Martínez-Rivera:** None. **L. González:** None. **C.S. Maldonado-Vlaar:** None.**Poster**

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.01/OO16

Topic: C.19. Behavioral Pharmacology

Support: NIH Grant MH086708

DOD CDMRP Autism Idea Award

Title: Targeting serotonin uptake to ameliorate social behavior deficiencies in pre-clinical models

Authors: *G. G. GOULD;
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Abstract: Impaired social behavior is a symptom that occurs in several psychiatric disorders including autism, schizophrenia and depression. Sociability impairments manifest in several forms, including indifference to social engagement, anxiety and/or empathy deficits. These dimensions, especially indifference to engagement, have proven difficult to treat with available pharmaceuticals. Based on clinical findings and experiments in rodents, serotonin (5-HT) neurotransmission is often disrupted in the socially-impaired brain, and the frontal cortex may be the main brain region involved. Two drugs are commonly used to treat autism; risperidone curbs aggression but blunts other forms of social interaction, and fluoxetine improves social behavior somewhat but is uneffective in individuals with reduced 5-HT transporter (SERT) function (due to common or rare gene polymorphisms). We have utilized two mouse models of impaired social behavior, inbred BTBR mice and SERT knock-out mice on a C57BL/6 background, to examine the effects of blocking SERT and novel drug targets on social behavior. In three-chambered sociability tests, social behavior of adult male BTBR mice worsened with 24h dietary tryptophan depletion, but improved significantly with 24h 5% dietary tryptophan supplementation ($p < 0.05$). BTBR sociability also improved with acute fluoxetine ($p < 0.05$), but not with citalopram treatment at doses ranging from (0.5 - 50 mg/kg). These findings indicate that 5-HT neurotransmission may be relatively low in BTBR mice. Our goal is to characterize the effects of blocking ancillary transporters of serotonin, with lower affinity but greater capacity than the SERT to remove 5-HT from extracellular fluid. These auxillary transporters, collectively known as 'uptake 2' include dopamine and norepinephrine transporters, as well as organic cation transporters and plasma membrane monoamine transporters found throughout the brain. The pseudoisocyanine decinium-22 (D-22) blocked 5-HT uptake in vitro (Km = 92 +/- 12 nM) but had negligible affinity for the SERT (Ki > 3000 nM) in hippocampal synaptosomes. In SERT -/- and BTBR mice, social behavior improved with acute D-22 treatment at doses of 0.01 - 0.1 mg/kg. This indicates that uptake 2 blockade may be an effective treatment for inpaired social behavior, and warrants further study. Our findings also support the idea that insufficient 5-HT neurotransmission may underlie impaired social behavior in autism and other psychiatric disorders.

Disclosures: G.G. Gould: None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.02/OO17

Topic: C.19. Behavioral Pharmacology

Support: Fondation Jerome Lejuene

Fédération pour la Recherche sur le Cerveau

IBRO

Title: Serotonin regulates hippocampal synaptic plasticity and object memory in mice

Authors: *S. P. FERNANDEZ[1], A. GRUART[2], J. DELGADO[2], P. GASPAR[1];
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Abstract: Low levels of serotonin (5-HT) have been associated with the learning and memory deficits seen in Alzheimer's disease, autism and major depression. Studies performed in healthy volunteers have shown that 5-HT depletion disrupts consolidation of new information, specifically in tasks involving delayed recall and/or recognition of visually-presented words, spoken words, pictures or abstract figures. Despite this evidence, the mechanism by which 5-HT regulates learning and memory function is not clear.
We used a genetic model of 5-HT depletion, Pet1 knock-out mice (Pet1 KO), to understand the role that 5-HT serves in modulating learning and memory function. In Pet1 KO mice, the levels of 5-HT are highly decreased, specifically associative cortical areas and the hippocampal formation are completely depleted of 5-HT axons. Our results showed that Pet1 KO mice are capable of acquiring associative fear conditioning, reward reinforced associative learning and motor learning; however, they do not recall familiarization with objects. The novel object recognition test was used to measure hippocampal-dependent declarative memory. The animals are trained to familiarize with two identical objects, and then subjected to a test session where one object is replaced by a novel one. This observation strongly linked object recognition deficit with 5-HT depletion; however does not specify the underlying mechanism. Previously it was shown that the consolidation of object memory involves the enhancement of synaptic strength across the CA3-CA1 hippocampal connexion; a phenomenon called as long-term potentiation (LTP). To test the hypothesis that LTP deficit is responsible for the memory impairment observed in Pet1KO mice, we performed in vivo recordings of field potentials in the CA1 region of the hippocampus of mice while performing the object memory task. We found that in Pet1 KO, experience-dependent LTP is exaggerated compared to wild-type mice, possibly explaining the aberration seen in forming correct maps of the object presented. These results established for the first time a direct link between central 5-HT depletion, memory impairment and synaptic plasticity deficits.

Disclosures: S.P. Fernandez: None. **A. Gruart:** None. **J. Delgado:** None. **P. Gaspar:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.03/OO18

Topic: C.19. Behavioral Pharmacology

Support: NIH Grant OD011132

NIH Grant DA015040

Title: Sub-chronic administration of MDMA leads to long term changes in murine social behavior

Authors: *D. W. CURRY[1,3], K. S. MURNANE[3], M. T. LOGUN[2], L. L. HOWELL[3,4]; [1]Neurosci. Grad. Program, [2]Emory Univ., Atlanta, GA; [3]Neuropharm. and Neurologic Dis., Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [4]Psychiatry and Behavioral Sci., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: S,R(+/-)-3,4-methylenedioxymethamphetamine (MDMA) is a ring substituted amphetamine derivative and popular recreational drug (ecstasy). It has robust prosocial effects that include increased gregariousness and empathy. Recently there has been a resurgence of interest in the drug's therapeutic potential, and several clinical trials have been undertaken to ascertain its efficacy for treating a variety of conditions including post-traumatic stress disorder and end of life anxiety. However the long term outcomes following MDMA use are decidedly mixed. While some users have reported improved social functioning and generally positive effects, others report increased depression, anxiety, and aggressive behavior. The neural changes that underpin these behavioral outcomes are not well understood. In this study, male Swiss Webster mice received a sub-chronic regimen of either racemic MDMA (7.8 mg/kg) or saline. Treatments were given via intraperitoneal injection every 48 hours for a total of 4 drug administrations. 30 minutes after each injection, mice were tested with a novel conspecific in a social interaction test. We found that mice sensitized to the prosocial effects of the drug, steadily increasing their social behavior following each subsequent drug administration. After an abstinence period of 48 hours, subjects were tested without drug, to assess any long term changes to baseline social behavior. Mirroring the human data, there was considerable variability in the long term effects of MDMA on murine behavior. There were increases in both social interaction and aggression, indicating within species variability to the drug's long term effects. Following testing, subjects were euthanized and their brains were analyzed for changes in monoamine content and serotonin receptor densities. These results were correlated with the various behavioral outcomes. The acute prosocial effects of MDMA appear to be conserved across species and our results indicate that even the long term effects on behavior can be investigated

using a mouse model. These findings will serve as the basis for future experiments that can determine the mechanisms underlying these behavioral changes. Because MDMA is both a widely used drug of abuse and a potential therapeutic, there is great impetus to better understand it's acute and long term behavioral and neurological effects. Furthermore, an understanding of these effects will provide significant insight into the neurobiological mechanisms that regulate and motivate social interaction.

Disclosures: D.W. Curry: None. **K.S. Murnane:** None. **M.T. Logun:** None. **L.L. Howell:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.04/PP1

Topic: C.19. Behavioral Pharmacology

Support: NIH Grant DA034488

NIH Grant DA06511

NIH Grant DA024157

NIH Grant DA000403

NIH Grant DA07287

Title: A predisposition toward inherent impulsivity is associated with elevated 5-HT2AR expression

Authors: *L. H. FINK[1], N. C. ANASTASIO[1], R. G. FOX[1], F. G. MOELLER[2], K. A. CUNNINGHAM[1];
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Abstract: Inherently poor response inhibition, or "action without reflection," may increase susceptibility to drug abuse and dependence. Serotonin (5-HT) systems play a nuanced role in impulsive action, perhaps mediated by 5-HT receptors in the prefrontal cortex (PFC). Selective 5-HT2A receptor (5-HT2AR) antagonists (e.g., M100907) reduce impulsive action with notable potency and efficacy, suggesting that tonic 5-HT2AR signaling supports impulsive behavior. We sought to test the hypothesis that the inherent predisposition to impulsive action is associated

with elevated 5-HT2AR expression and function in the PFC. These studies employed the one-choice serial reaction time (1-CSRT) task to identify high (HI) and low (LI) impulsive outbred rats. Rats were trained to nose-poke to receive a food reinforcer on a 5-sec inter-trial interval (ITI) schedule; responses during the ITI (premature responses) resulted in further delays of reward presentation. The upper 25% and lower 25% of animals were identified as HI or LI rats, respectively. Rats were sacrificed and the medial PFC (mPFC) was harvested, and crude synaptosomal protein extracted for western blot analysis. In separate sets of animals, the ability of M100907 (0.003, 0.01, 0.03, 0.1 mg/kg, i.p.) to suppress premature responses or the ability of the 5-HT2AR agonist DOI (1 mg/kg, s.c.) to elicit the head twitch response was evaluated in HI and LI rats. HI rats displayed higher 5-HT2AR expression in crude synaptosomal fractions of the mPFC relative to LI rats ($p<0.05$, Student's t-test). Higher doses of M100907 (0.03, 0.1 mg/kg) suppressed premature responses in all rats, but lower doses (0.003, 0.01 mg/kg) suppressed premature responses selectively in HI, but not, LI rats ($p<0.05$, Dunnett's test). DOI elicited a greater head twitch response in HI relative to LI rats ($p<0.05$, Student's t-test). These data demonstrate that high impulsive action is associated with elevated expression and enhanced function of the 5-HT2AR, suggesting that differential 5-HT2AR function in the mPFC may in part drive high and low impulsive action.

Disclosures: L.H. Fink: None. **N.C. Anastasio:** None. **R.G. Fox:** None. **F.G. Moeller:** None. **K.A. Cunningham:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.05/PP2

Topic: C.19. Behavioral Pharmacology

Support: NARSAD Young Investigator

Title: Fast onset of action and neurogenesis dependency of 5HT4 receptor agonist in an animal model of anxiety/depression

Authors: *I. DAVID[1], D. J. DAVID[1], Z. EL-ALI[2], F. DARCET[1], M. WU[3], S. KERDINE[2], R. HEN[3], A. M. GARDIER[1,3];
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Abstract: Current antidepressants, as the selective serotonin reuptake inhibitor (SSRI) fluoxetine, have proven effective for the treatment of anxiety and depression. A relatively slow onset to therapeutic response of current antidepressants, combined with one third of patients who do not respond to first treatment makes it evident that the discovery of targets providing fast-acting antidepressant activity is desirable. It has been recently suggested that activation of 5-HT4 receptors might be a new strategy for developing faster-acting antidepressants. However, the precise mechanism through how this activation induces antidepressant response remains elusive. Here, we demonstrate using a mouse model of anxiety/depression, that a chronic treatment with the 5-HT4 receptor partial agonist RS67333 (1.5 mg/kg/d for x days), similarly to chronic fluoxetine (18mg/kg/d), induces anxiolytic/antidepressant-like activity in various behavioural tests and also stimulates adult hippocampal neurogenesis, Moreover, a chronic treatment with the 5-HT4 receptor antagonist GR125487, prevents the anxiolytic/antidepressant-like activity and the neurogenic effects of fluoxetine, pointing out the relationship between 5-HT4 receptors activation and antidepressant activity. Interestingly, in this mouse model of anxiety/depression and in contrast to fluoxetine, a fast acting anxiolytic/antidepressant activity of RS67333 is also observed up to 7-days. Furthermore, using this mouse model of anxiety/depression combined with an ablation of hippocampal neurogenesis by X-irradiation, we found that both neurogenesis-dependent and -independent mechanisms are involved in the anxiolytic/antidepressant efficacy of a 5-HT4 receptor agonist. Finally, we identified β-arrestin 1 as a putative blood biomarker in mouse leukocyte predicting fast response to antidepressants.
These results point out toward a pharmacological approach in which the activation of postsynaptic 5-HT4 receptors by its agonist provides an antidepressant like response that emerges faster than traditional SSRI antidepressants. This fast onset of action is unlikely recruiting adult hippocampus.

Disclosures: I. David: None. **D.J. David:** None. **Z. El-ali:** None. **S. Kerdine:** None. **F. Darcet:** None. **A.M. Gardier:** None. **M. Wu:** None. **R. Hen:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.06/PP3

Topic: C.19. Behavioral Pharmacology

Support: MEXT, Grant-in-Aid for Scientific Research (A) (21240031)

MEXT, Grants-in-Aid for Scientific Research (B) (24700342)

Title: Rines E3 ubiquitin ligase regulates MAO-A levels and emotional responses

Authors: *M. KABAYAMA[1], K. SAKOORI[2], K. YAMADA[3], M. ODAGAWA[2], N. MORIMURA[2], K.-I. KATAYAMA[5], N. P. MURPHY[4], J. ARUGA[2];
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Abstract: Monoamine oxidase A (MAO-A), the catabolic enzyme of norepinephrine and serotonin, plays a critical role in emotional and social behavior. However, the control and impact of endogenous MAO-A levels in brain remains unknown. Here we show that the RING finger-type E3 ubiquitin ligase, Rines/RNF180 regulates brain MAO-A subset, monoamine levels and emotional behavior. Rines interacted with MAO-A and promoted its ubiquitination and degradation. Rines knockout mice displayed impaired stress responses and enhanced anxiety and affiliative behavior. Norepinephrine and serotonin levels were altered in locus ceruleus, prefrontal cortex, and amygdala in either stressed or resting condition and MAO-A enzymatic activity was enhanced in the locus ceruleus in Rines knockout mice. Treatment of Rines knockout mice with MAO inhibitors showed genotype-specific effects on some of the affective abnormal behaviors. These results indicated that the emotional behavior control by Rines is partly due to the regulation of MAO-A levels. Collectively, these findings verify that Rines is a critical regulator of the monoaminergic system and emotional behavior and identify a promising candidate drug target for treating diseases associated with emotion.

Disclosures: M. Kabayama: None. **K. Sakoori:** None. **K. Yamada:** None. **M. Odagawa:** None. **N. Morimura:** None. **K. Katayama:** None. **N.P. Murphy:** None. **J. Aruga:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.07/PP4

Topic: C.19. Behavioral Pharmacology

Support: Autism Science Foundation Predoctoral Fellowship

NIH Grant HD071998

Title: Fluoxetine exposure during development affects later social behavior in the prairie vole (Microtus ochrogaster)

Authors: *R. H. LARKE, K. L. BALES;
Psychology, Univ. of California, Davis, Davis, CA

Abstract: Prenatal serotonin system manipulation is known to alter social behavior, and has been linked to the development of autism spectrum disorder. In this study, the prairie vole (Microtus ochrogaster), a rodent which forms lasting pair-bonds, was used as a model to test the effects of prenatal selective serotonin reuptake inhibitor (SSRI) exposure on social behavior and attachment formation. Twenty pregnant female prairie voles were treated with 5mg/kg subcutaneous fluoxetine or saline vehicle daily from the birth of their second litter to birth of the fourth litter. This allowed for 3 treatment groups: prenatal exposure; postnatal exposure; pre- and postnatal exposure. One male and one female from each litter were tested behaviorally during periadolescence, and another male and female were tested as adults. Animals were housed with an age, sex, and treatment matched animal prior to behavioral testing. Behavioral tests were chosen to detect social deficits and changes in anxiety-like behavior, and included open field, elevated plus maze, alloparental care, juvenile affiliation, intrasexual aggression, and partner preference tests. Preliminary analyses indicate no effect of SSRI exposure on anxiety-like behavior during periadolescence, but do indicate an effect on alloparental behavior during this period. Grant funding by: Autism Science Foundation, HD071998

Disclosures: R.H. Larke: None. **K.L. Bales:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.08/PP5

Topic: C.19. Behavioral Pharmacology

Title: Effects of a sertraline (an SSRI antidepressant) and venlafaxine (an SNRI antidepressant) on forced swim test behavior and neurogenesis levels in female rats

Authors: *J. KOTT, S. BRUMMELTE;
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Abstract: Depression is twice as prevalent in women as in men and the highest risk for women to develop a depressive episode is during her reproductive years. However, less research has been done to study the effects of antidepressants in females. In males, anti-depressants often increase neurogenesis in the dentate gyrus. This study aims to investigate the effects of two different antidepressants, sertraline (Zoloft, a selective serotonin reuptake inhibitor, SSRI) and venlafaxine (Effexor, a serotonin/norepinephrine reuptake inhibitor, SNRI) on depressive-like

behavior and hippocampal neurogenesis levels in female rats. For this, all animals are first tested in the forced swim test (FST), to determine their 'baseline' depressive-like behavior before oral administration of either sertraline (10 and 20mg/kg), venlafaxine (10 and 20mg/kg) or saline begins and continues for 14 days. On the last day of administration (d14), animals are again tested in the FST and are sacrificed via perfusion the following day (d15), to investigate neurogenesis levels in the dentate gyrus. We hypothesize that the antidepressants will be effective in altering forced swim test behavior in female rats, but will have less of an impact on neurogenesis levels compared to previous reports in males. This study will help us to further our understanding of the association between depressive-like behavior, antidepressants, and neurogenesis in females.

Disclosures: J. Kott: None. **S. Brummelte:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.09/PP6

Topic: C.19. Behavioral Pharmacology

Title: Roles of serotonin during the postnatal period in the anxiety, depression, and the spatial learning in adult BALB/c mice

Authors: *C. ISHIKAWA[1], A. OHTANI[1], M. YOSHIKAWA[2], T. SHIGA[1];
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Abstract: Serotonin (5-hydroxytryptoamine, 5-HT) is known to act as a neurotransmitter in the adulthood and a neurotrophic factor in the brain development. We have previously reported that the oral administration of fluoxetine, selective serotonin reuptake inhibitor (SSRI) during postnatal weeks 1-3 normalized 5-HT turnover rate, dendritic spine and synapse densities, and spatial learning in prenatally stressed C57BL/6J mice (Ishiwata et al., 2005). Interestingly, the same treatment had no effect in no-prenatally stressed mice. These results suggest that adequate amount of 5-HT is important for the proper development of brain and behavior. However, the effect of SSRI depends on the animals tested and experimental paradigms, thus it is unclear. BALB/c mice is known to have less amount of brain 5-HT and be more anxious compared to other strains such as C57BL/6 and 129x/Sv. Therefore, we hypothesized that fluoxetine has some positive effects in BALB/c mice without stressing prenatally. In the present study, we examined the effects of SSRI on BALB/cCrSlc male mice by oral administration of fluoxetine (5 mg/kg BW/day) during postnatal weeks 1-3. We found that fluoxetine treatment increased the entries of

open arms significantly and the tendency to stay longer in open arms in elevated plus maze, suggesting the decrease of anxiety. In addition, fluoxetine treatment significantly improved the spatial learning in Morris water maze, and decreased depression-like behavior such as floating in forced swim test. In these mice, the 5-HT2A-receptor mRNA expression was increased and BDNF mRNA expression was the same in the frontal cortex. Thus, fluoxetine recovered the serotonergic system due to the insufficient 5-HT level in BALB/c mice. We are currently studying the roles of 5-HT receptors, which mediate the effects of 5-HT, using 5-HT1A-receptor agonist (8-OH-DPAT) and 5-HT2A-receptor antagonist (ketanserin).

Disclosures: C. Ishikawa: None. **A. Ohtani:** None. **M. Yoshikawa:** None. **T. Shiga:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.10/PP7

Topic: C.19. Behavioral Pharmacology

Title: A backtranslational study of the selective 5-HT2C agonist lorcaserin in two rat obesity models

Authors: *G. A. HIGGINS[1,2], J. DESNOYER[1], A. VAN NIEKERK[1], L. B. SILENIEKS[1], W. LAU[1], S. THEVARKUNNEL[1], J. DELAY[3], H. DOBSON[1,3];
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Abstract: The publication of multiple clinical reports detailing the clinical effects of the recently approved 5-HT2C agonist lorcaserin (LOR) in obese patients, provides opportunity to back-translate to preclinical obesity models. We have investigated the effect of LOR administered twice daily for 28 days in two rat obesity models, (1) the diet induced obesity (DIO) model, and (2) the Zucker rat model. In each study the model was first characterized based on body weight and composition using non-invasive Quantitative Magnetic Resonance (QMR) technology and plasma lipid profile, relative to controls, before drug treatment began. Primary efficacy endpoints were change in body weight, body fat composition (DIO study only), and plasma lipid profile. Secondary efficacy measures of food and water intake were also recorded. In the DIO study at the completion of the treatment phase, the effect of 28 day LOR treatment on cardiac function was assessed using echocardiography.
In the DIO study, 26 male Sprague-Dawley rats given ad-lib access to a high fat diet (Research Diets Inc., D12492, 60% kcal% fat) for 90 days had significantly greater body weight (703+12g

vs. 611+18g; P<0.01), % fat content (18.7+0.6% vs. 11.4+0.6%; P<0.01) and plasma lipid profile (e.g cholesterol: 3.21+0.12 vs. 2.36+0.11; P<0.01) compared to rats fed regular diet (LabDiet 5001, ~13% kcal% fat). Next, the DIO rats were divided into 3 groups allocated to receive either saline vehicle (SC b.i.d; N=10), LOR 1mg/kg (SC b.i.d; N=8), LOR 2mg/kg (SC b.i.d; N=8). Food and water intakes were measured daily and at the study completion both body composition and plasma lipid profile was remeasured. After 28 days treatment, LOR significantly affected each primary endpoint measure of body weight change (Veh: +11.3+0.5%; LOR 1: +7.8+0.1%; LOR 2: +5.9+0.6%; P<0.01), body fat percentage (Veh: +10.6+4.4%; LOR 1: 2.0+3.8%; LOR 2: -6.5+2.9%; P<0.01) and plasma cholesterol. Additional safety studies identified no evidence of liver or cardiac toxicity.
In the second study, 14 male Zucker rats received either saline vehicle or LOR (3mg/kg SC b.i.d) over 28 days. Food and water intakes were measured daily and plasma lipid profile was measured pre- and post-treatment. LOR reduced body weight gain (Veh: +0.9+1.4%; LOR 3: -3.4+2.6%; P<0.05) and multiple plasma lipid biomarkers, e.g cholesterol (Veh: 16.5+2.7, LOR 3: 10.3+1.4; P<0.05). Taken together the results of both studies show marked similarity to clinical findings with an approximate 5% change in body mass compared to control group, and modest yet reliable effects on lipid biomarkers and fat mass. LOR was also well tolerated at these doses, further consistent with clinical experience.

Disclosures: G.A. Higgins: None. **J. Desnoyer:** None. **A. Van Niekerk:** None. **L.B. Silenieks:** None. **W. Lau:** None. **S. Thevarkunnel:** None. **H. Dobson:** None. **J. Delay:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.11/PP8

Topic: C.19. Behavioral Pharmacology

Support: NIH Grant DA002925

Title: Characterizing the next generation of psychoactive designer drugs: behavioral pharmacology of synthetic cathinones and substituted phenethylamines

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Abstract: In recent years, a wide variety of synthetic psychoactive drugs have been sold by online vendors as "research chemicals" or "legal highs." Unfortunately, very little is known about the pharmacology and toxicology of these compounds. Examples of substances that are currently

being distributed include cathinone derivatives (components of "bath salts"), which release monoamines and have effects similar to amphetamine (AMPH) and 3,4-methylenedioxymethamphetamine (MDMA), and N-benzylphenethylamines such as N-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (25I-NBOMe), which act as 5-HT2A agonists and produce hallucinogenic effects. We have characterized the effects of these compounds in rodents using the Behavioral Pattern Monitor (BPM) and head twitch response (HTR). The BPM quantifies locomotor and investigatory responses, and can distinguish between the effects of hallucinogens, AMPH-like stimulants, and MDMA-like drugs. Administration of the cathinone derivative methylone (3,4-methylenedioxymethcathinone; 2.5-10 mg/kg, IP) to Sprague-Dawley rats increased locomotor activity, reduced measures of investigatory behavior, and induced thiogmotaxis. A similar pattern of effects occurs with MDMA, but not with AMPH or hallucinogens. As with MDMA, the locomotor hyperactivity induced by methylone was attenuated by pretreatment with the serotonin reuptake inhibitor fluoxetine (10 mg/kg, IP). The HTR is a rhythmic paroxysmal rotational head movement that occurs in rodents in response to 5-HT2A activation. We assessed the HTR using a head-mounted magnet and a magnetometer coil, which can detect the behavior with high sensitivity. 25I-NBOMe produced a dose-dependent increase in HTR counts in C57BL/6J mice and was highly potent (ED50 = 0.078 mg/kg, SC). Additionally, the selective 5-HT2A antagonist M100907 completely blocked the HTR induced by 25I-NBOMe. Thus, methylone appears to produce an MDMA-like profile of behavioral changes by virtue of releasing presynaptic serotonin, whereas 25I-NBOMe induces the HTR via 5-HT2A receptor activation, consistent with its classification as a hallucinogen. These findings demonstrate that it is possible to characterize the behavioral effects of novel psychoactive substances using acute, unconditioned behavioral assays in rodents. Future studies will characterize the effects of a wider range of designer drugs using these behavioral paradigms.
Supported by: NIDA (DA002925)

Disclosures: A.L. Halberstadt: A. Employment/Salary (full or part-time):; University of California San Diego. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Brain & Behavior Research Foundation. **S.B. Powell:** A. Employment/Salary (full or part-time):; UCSD. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Servier, NIMH, and the U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center (MIRECC) and Center of Excellence in Stress and Mental Health (CESAMH). **M.A. Geyer:** A. Employment/Salary (full or part-time):; UCSD. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Intracellular Therapeutics, Johnson & Johnson, NIDA, NIMH, and the U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical

Center.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); San Diego Instruments. F. Consulting Fees (e.g., advisory boards); Abbott, Acadia, Addex, Cerca, Lundbeck, Merck, Neurocrine, Omeros, Takeda, Teva.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.12/PP9

Topic: C.19. Behavioral Pharmacology

Support: K05 DA020087 (KAC)

R21 MH093844 (JZ/KAC)

Welch Foundation Chemistry and Biology Collaborative Grant from Gulf Coast Consortia (GCC) for Chemical Genomics

John Sealy Memorial Endowment Fund

Title: Pharmacological evaluation of novel positive allosteric modulators of serotonin 2C receptor

Authors: *G. ZHANG[1], C. DING[2,3], N. C. ANASTASIO[1,3], J. S. MONCRIEF[1], T. M. CARBONARO[1,3], R. G. FOX[1,3], S. J. STUTZ[1,3], T. D. SMITH[1,3], C. WILD[2,3], J. ZHOU[1,2,3], K. A. CUNNINGHAM[1,3];
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Abstract: The serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) is implicated in a diversity of physiological functions and neural disorders. Allosteric modulators of the 5-HT_{2C}R present a unique drug design strategy. We hypothesize that small molecule positive allosteric modulators (PAMs) of 5-HT_{2C}R will be a novel pharmacological means to selectively enhance 5-HT_{2C}R function *in vitro* and *in vivo* as potential pharmacotherapy with an acceptable side effect profile. We have successfully identified two synthetic routes for PNU-69176E, the only reported selective PAM for the 5-HT_{2C}R. We utilized PNU-69176E as the chemical lead to optimize the polar head domain, the lipophilic binding site, and the core scaffold to design and synthesize novel drug-like small molecules with high potency and selectivity for the 5-HT_{2C}R over the highly homologous 5-HT_{2A}R. Structure-activity relationships (SARs) were established through

radioligand binding as well as efficacy to modulate 5-HT_{2C}R-mediated downstream effectors in cell signaling assays upon exposure to orthosteric ligands (e.g., 5-HT, 5-HT_{2C}R agonist WAY163909) in Chinese Hamster Ovary (CHO) cells stably transfected with the 5-HT_{2C}R (5-HT_{2C}R-CHO cells) or 5-HT_{2A}R (5-HT_{2A}R-CHO cells). Several analogues (e.g., CYD-1-79 and CYD-6-16-2) potentiated 5-HT and WAY163909-evoked intracellular calcium (Ca_i^{++}) release in 5-HT_{2C}R-CHO, but not 5-HT_{2A}R-CHO, cells. These compounds also induced a leftward shift in the 5-HT concentration-response curve in 5-HT_{2C}R-CHO cells. CYD-1-79 and CYD-6-16-2 exhibited favorable *in vivo* bioavailability (39.1% and 27.6%, respectively) and half-life (~6 hrs and 1.5 hrs, respectively). CYD-1-79 dose-dependently modulated 5-HT_{2C}R-associated behaviors (e.g., spontaneous locomotor activity, self-grooming, impulsive action) in Sprague-Dawley rats. Taken together, our *in vitro* and *in vivo* data identified novel, selective 5-HT_{2C}R PAMs. These unique small molecules open new avenues for probing the chemical neurobiology of 5-HT_{2C}R allosteric modulation and developing novel pharmacotherapeutics for neural disorders characterized by deficient 5-HT_{2C}R function.

Disclosures: G. Zhang: None. **C. Ding:** None. **N.C. Anastasio:** None. **J.S. Moncrief:** None. **T.M. Carbonaro:** None. **R.G. Fox:** None. **S.J. Stutz:** None. **T.D. Smith:** None. **C. Wild:** None. **J. Zhou:** None. **K.A. Cunningham:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.13/PP10

Topic: C.19. Behavioral Pharmacology

Support: NIH Grant K99 DA033374

NIH Grant K05 DA020087

The Klarman Family Foundation

Title: Stop, put that cookie down: Impulsive action and binge intake of palatable food

Authors: *N. C. ANASTASIO, S. J. STUTZ, K. A. CUNNINGHAM;
Ctr. for Addiction Research, Dept. Pharmacol. and Toxicology, Univ. Texas Med. Br., Galveston, TX

Abstract: Food intake is essential for survival, but maladaptive patterns of intake, possibly encoded by a preexisting vulnerability coupled with the influence of environmental variables, can modify the reward value of food. Impulsivity, a predisposition toward rapid unplanned reactions to stimuli, is one of the multifaceted determinants underlying the etiology of dysregulated eating, its evolving pathogenesis, and treatment outcomes. Impulsivity and dysregulated eating converge mechanistically at the level of serotonin (5-HT) neurotransmission at the 5-HT2A receptor (5-HT2AR) and 5-HT2CR within an integrated brain network (e.g., prefrontal cortex, nucleus accumbens), that orchestrates a balance between stimulus-driven and goal-driven behaviors. Disturbances in this system may engender maladaptive eating behaviors [esp., binge eating on palatable high fat/sugar ("sweet-fat) foods] and the response to food stimuli seen in binge eating disorder. Yet, our understanding of the reciprocal relationships linking impulsivity to binge eating and/or relapse in the presence of food stimuli, and the shared neurobiological mechanisms, is very limited. We developed a rat model of binge eating behavior in which "binge" rats allowed unrestricted 2-hr access to sweet-fat chow (17% sucrose and 45% fat by kCal) at the start of the dark cycle consume significantly greater calories relative to control rats maintained on brown chow or continual sweet-fat chow. The highly-selective and efficacious 5-HT2CR agonist WAY163909 suppresses binge intake of sweet-fat chow ($p<0.05$) at doses lower than those required to suppress brown chow intake. To explore the relationship between impulsivity and binge-eating, we identified high (HI) and low impulsive (LI) rats in a novel model of impulsive action (1-choice serial reaction time task) in which a sweet-fat food pellet was the reinforcer. HI rats repeatedly exhibited significantly higher bingeing on sweet-fat chow compared to LI rats ($p<0.05$); a positive correlation was observed between levels of impulsivity and caloric intake during the binge ($r=0.457$, $p<0.05$). Elimination of the 5-HT2CR in the nucleus accumbens resulted in high binge eating, high impulsivity, and a shift in functional tone of the homologous 5-HT2AR. Thus, inherent impulsivity and binge eating reciprocally interact at the level of the 5-HT2R to control behavior. Through addressing a fundamental gap in our knowledge of how the neural and behavioral aspects of impulsive action are related to binge eating, we hope to develop pharmacological strategies to minimize binge eating and enhance clinical practice for disorders of overeating.

Disclosures: N.C. Anastasio: None. **S.J. Stutz:** None. **K.A. Cunningham:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.14/PP11

Topic: C.19. Behavioral Pharmacology

Support: NSERC

AIHS

Title: Prenatal exposure to fluoxetine results in behavioral and anatomical deficits in offspring; Does tactile stimulation reduce the observed impairments?

Authors: *R. L. GIBB[1], A. NAKAHASHI[2], D. O. FROST[3], B. E. KOLB[2];
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Abstract: Fluoxetine is an antidepressant widely used in a variety of populations including women with perinatal depression. However, the effect of prenatal selective serotonin reuptake inhibitor exposure has not been fully understood. Previously, our lab showed that daily oral administration of fluoxetine on normal pregnant rats produces adverse behavioural effects on offspring. In the current study, we attempted to replicate the previous findings using a different drug administration method, and introduced a possible remediating factor. In order to better mimic human cases of fluoxetine administraion, instead of giving a daily single dose, we replaced the drinking water with drug solution and chronically exposed the dams to fluoxetine. Following the birth of pups, tactile stimulation (TS) was given in a cross-litter design, which is a well-established favourable experience. Female offspring exposed to prenatal fluoxetine exhibited de-feminized traits in anxiety- and exploratory-related behavioural tests. Prenatal fluoxetine impaired Morris Water Task performance in males, while the male offspring given the combination of prenatal fluoxetine and postnatal TS performed superiorly compared to all other groups. Anatomically, female fluoxetine offspring showed a reduction in cortical thickness and posterior thalamic size regardless of TS, while males showed a diminishing effect of prenatal fluoxetine in the increase in anterior thalamic size. Fluoxetine rats weighed less than control rats throughout the experiment, and TS did not remediate this effect. These preliminary results suggest that fluoxetine adversely affects offspring outcome even in the absence of depressive traits in the mothers and TS changes behaviour and brain.

Disclosures: R.L. Gibb: None. **A. Nakahashi:** None. **D.O. Frost:** None. **B.E. Kolb:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.15/PP12

Topic: C.19. Behavioral Pharmacology

Support: FNRS-FRS

Title: A non-invasive method of fluoxetine administration to rats

Authors: J. PAWLUSKI[1,3], E. VAN DONKELAAR[3], Z. ABRAMS[4], V. HOUBART[2], H. STEINBUSCH[3], M. FILLET[2], *T. D. CHARLIER[4];
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Abstract: Selective serotonin reuptake inhibitor (SSRI) medications are one of the most common treatments for mood disorders. In humans, these medications are taken orally, usually one time per day. Unfortunately, administration of antidepressant medications in rodent models is often through injection, oral gavage, or minipump implant, all relatively stressful procedures. Recent research in animal models investigating effects of environmental teratogens on development, has demonstrated that administration of a solution injected in a wafer cookie may be just as effective as injecting a solution into the animal, and thus would reduce the stress to the animal, particularly for long-term daily treatment (greater than 4 weeks). However, it remains to be determined whether this method of drug administration is effective for antidepressant medications and is comparable to other methods of antidepressant administration that are commonly used. Therefore, the aim of this project was to investigate how administration of the commonly used SSRI, fluoxetine, via a wafer cookie (Croustifondante, Delacre), compares to fluoxetine administration using an osmotic minipump (Alzet). For this experiment, adult female Sprague-Dawley rats were divided over the two administration methods; 1) Cookie and 2) Osmotic minipump, and three fluoxetine treatment doses; 0, 5 or 10mg/kg/day. Blood collection was taken at set time points in order to determine how these treatment methods affect drug levels and metabolism throughout the day. Results show that a fluoxetine dose of 5mg/kg/day results in comparable serum levels of fluoxetine and its active metabolite norfluoxetine between the two administration methods. However, minipump administration of 10mg/kg/day dose of fluoxetine resulted in significantly elevated levels of fluoxetine and norfluoxetine compared to the same dose administered once per day via a cookie. Hippocampal cell proliferation was also assessed in these females after 2 weeks of treatment and preliminary results show no marked difference in proliferation after 2 weeks of fluoxetine administration, regardless of administration method or fluoxetine dose. Together this data suggest that a once daily cookie administration of fluoxetine at a 5mg/kg/day dose is as effective as using an osmotic minipump with the same fluoxetine dose.

Disclosures: J. Pawluski: None. **T.D. Charlier:** None. **E. van Donkelaar:** None. **Z. Abrams:** None. **H. Steinbusch:** None. **V. Houbart:** None. **M. Fillet:** None.

Poster

547. **Monoamines and Behavior: Serotonin and Histamine**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 547.16/PP13

Topic: C.19. Behavioral Pharmacology

Support: Tata Institute of Fundamental Research Intramural Grant (VAV)

Title: Early life perturbations of 5HT2 receptor lead to disrupted anxiety related behavior in adulthood

Authors: *P. CHACHRA, A. SARKAR, V. A. VAIDYA;
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Abstract: The temporal window of postnatal life is critical for defining the trajectory of emotional development. Evidences indicate that during early life, environmental and pharmacological perturbations evoke lasting and robust effects on the development of mood-related behavior. Serotonin neurotransmission in early life has been reported to influence the development of emotionality. In early life, 5-HT2A/2C receptors contribute to 5HT mediated excitatory cortical responses and genetic knockout studies implicate the role of 5-HT2A and 5-HT2C receptors in the regulation of anxiety. In addition, recent reports demonstrate that models of psychiatric susceptibility based on adverse early life experience, including maternal separation and prenatal exposure to influenza exhibit potentiated 5-HT2A receptor mediated functional responses in adulthood. These evidences provided impetus for our study, which was aimed at examining the effects of chronic postnatal 5HT2 receptor stimulation, using the partial 5-HT2A/2C agonist l-(2,5-dimethoxy-4-iodophenyl)2-aminopropane (DOI), on adult anxiety behavior and the underlying molecular mechanisms.

Our findings reveal that postnatal stimulation of 5HT2A/2C receptors from postnatal day 2-21 is sufficient to evoke anxiogenic responses in adulthood in rats on the elevated plus maze (EPM) and open field tests (OFT). In the EPM task, postnatal DOI treatment (PNDOI) in animals, resulted in significant decrease in the percent path length in the open arms of the maze and a significant increase in percent path length in the closed arms in adulthood. In the OFT, PNDOI animals showed a significant decline in the percent path length traversed in the centre and the percent time spent in the centre of the arena. Experiments are underway to characterize the underlying molecular changes within limbic brain regions implicated in the regulation of mood, in PNDOI animals.

It is of interest that the enhanced anxiety states, observed following chronic postnatal DOI treatment differ from the anxiolytic effects reported earlier with acute adult DOI administration and no effects reported with chronic DOI administration in adulthood. Our results suggest that during this early time window, 5-HT2A/C receptors might be involved in the establishment of adult anxiety.

Disclosures: P. Chachra: None. **A. Sarkar:** None. **V.A. Vaidya:** None.**Poster**

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.01/PP14

Topic: D.02. Auditory

Support: NHMRC APP1052463

Title: Type II spiral ganglion neurons contribute to contralateral suppression of hearing

Authors: *K. E. FROUD[1], A. C. Y. WONG[1], M. KLUGMANN[1], J.-P. JULIEN[2], A. F. RYAN[3,4], G. D. HOUSLEY[1];
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Abstract: A major unanswered question in auditory neuroscience is how sensory input drives contralateral suppression (CS), the reduction in hearing sensitivity in one ear when sound is delivered to the opposite ear. The decline in hearing sensitivity arises from inhibition of auditory transduction in the cochlear outer hair cells (OHCs) mediated by the medial olivocochlear (MOC) efferent pathway. MOC neurons originate in the superior olivary complex in the brainstem. In response to sound in the contralateral ear, these neurons are activated, and via α9 / α10 nicotinic acetylcholine receptor synapses, the OHCs in the ipsilateral cochlea are hyperpolarized, increasing their thresholds. The MOC neurons must be activated via sensory input from the contralateral cochlea. Therefore either type I and / or type II spiral ganglion neurons (SGN) are involved. The type I SGN (95% of the cochlear sensory fibres) synapse with inner hair cells, while type II SGN (the remaining 5%) innervate the OHCs. Our experiments utilized a peripherin (Pph-/-) knockout mouse model to investigate the possibility that type II SGN contribute to CS. Pph is a type III intermediate filament expressed only by type II SGN, and loss of Pph expression affects the development of these neurons [1]. We therefore tested whether loss of Pph expression affected type II SGN innervation of OHC, and if so, whether this was associated with altered CS. OHC innervation was compared in Pph-/- versus WT control mice (C129/Bl6 strain) using CtBP2 / Ribeye immunofluorescence to identify the number and location of ribbon synapses.Cochlear mid-modiolar cryosections from adult (~ 4 months old) Pph-/- and WT mice were batch processed and imaged using confocal microscopy. The location of CtBP2-immunolabeled afferent synapses was recorded relative to the centre of the OHC nucleus. This analysis revealed apical displacement of synapses on the Pph-/- OHCs. Quadratic (f2-f1) Distortion Product Otoacoustic Emissions (DPOAEs) are decreased by CS [2]. We

therefore measured the effect of sound delivered to the contralateral ear, on the quadratic DPOAEs (in the ipsilateral ear) of Pph-/- and WT mice. These experiments showed that while the quadratic DPOAE magnitudes were the same in Pph-/- and WT mice, they were significantly less attenuated by contralateral sound in Pph-/- animals. This identifies a significant contribution of the type II SGN to the sensory signal driving the MOC pathway to the OHCs in the opposite cochlea. The experiments were conducted with University of New South Wales Animal Care and Ethics Committee approval.

1.Barclay, M., et al., Neural Dev, 2011. 6: p. 33.
2.Abel, C., et al., J Neurophysiol, 2009. 101(5): p. 2362-71.

Disclosures: K.E. Froud: None. **A.C.Y. Wong:** None. **M. Klugmann:** None. **J. Julien:** None. **A.F. Ryan:** None. **G.D. Housley:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.02/PP15

Topic: D.02. Auditory

Support: Howard Hughes Medical Institute

Title: Zebrafish sensory hair-cell activity is facilitated by dopaminergic efferents via the D1 dopamine receptor

Authors: *C. G. PHILLIPS[1], J. G. TRAPANI[2], T. NICOLSON[1];
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Abstract: Dopamine (DA) is an important neuromodulator throughout the nervous system. The effects of DA have been well-characterized in many regions including the retina where it is released in a tonic, paracrine fashion and regulates multiple aspects of vision. Dopaminergic neurons innervate hearing and balance organs, yet the influence of DA on auditory and vestibular sensory cells is not fully understood. Some hypothesize DA action on the postsynaptic primary afferent terminal, while others hypothesize direct action on hair cells. Here we show the influence of DA on the primary sensory hair-cells of the zebrafish lateral line organ using in vivo imaging, whole-mount immunocytochemistry and electrophysiological recordings. The lateral line is composed of neuromasts: small islands of hair cells that project cilia out from the fish to sense water movement, analogous to hair cells of the mammalian inner ear. Using an antibody against the zebrafish D1 dopamine receptor (D1R) we demonstrate that D1R is expressed by hair cells. D1Rs colocalize with vesicular glutamate transporter 3 (vGlut3, expressed on synaptic

vesicles) and ribeye, the main component of the synaptic ribbon. Using extracellular microphonics recordings, we show that DA facilitates the activity of hair cells via the D1R-pathway. We show the innervation pattern of dopaminergic efferents using transgenic zebrafish that express EGFP under the control of the DA transporter promoter (Tg[dat:EGFP]). By crossing Tg[dat:EGFP] fish with other transgenics that express fluorophores specifically in hair cells (Tg[myo6b:TdTomato]) or afferent neurons (Tg[neuroD:TdTomato]) we conclude that dopaminergic neurons are unlikely to directly synapse onto either cell-type. Additionally, using an antibody against the acetylcholine transporter (ChAT) and a marker for presynaptic terminals (synaptophysin) we show that cholinergic efferents and dopaminergic efferents do not colocalize. Overall our data suggest that lateral line efferents release DA in a tonic and paracrine fashion, and that DA binds to D1Rs on hair cells which leads to increased hair-cell activity.

Disclosures: C.G. Phillips: None. **J.G. Trapani:** None. **T. Nicolson:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.03/PP16

Topic: D.02. Auditory

Support: NIH Grant DC10000

Title: Presynaptic release properties show target-specific regulation at auditory nerve terminals in avian cochlear nuclei

Authors: J. AHN, *K. M. MACLEOD;
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Abstract: Short-term synaptic plasticity acts as a time- and firing rate-dependent filter that mediates the transmission of information across synapses. In the auditory brain stem, short-term depression and facilitation are expressed in a target-dependent manner at the synaptic connections between auditory nerve fibers and the neurons in the two cochlear nuclei, nucleus magnocellularis (NM) and nucleus angularis (NA). This differential plasticity contributes to the divergence of acoustic timing and intensity information into parallel pathways. In NA, the beginning of the intensity pathway, the plasticity consists of a mixture of facilitation and depression, while the synaptic plasticity of inputs to NM in the timing pathway is dominated by short-term depression. We investigated how presynaptic mechanisms of transmitter release might differ in the two pathways. We characterized the pharmacological sensitivity of calcium channels underlying release and estimated the relative release probability using whole-cell voltage clamp

recordings in chick auditory brain stem slices.
Excitatory postsynaptic currents (EPSCs) evoked by extracellular stimulation of auditory nerve fibers and recorded in NA neurons were blocked >90% with bath application of ω-conotoxin, implicating N-type channels, while ω-agatoxin caused partial blockade. These results agree with published results from NM (Sivaramakrishnan & Laurent, 1999) suggesting identical calcium channel types underlie synaptic transmission in both nuclei. Next, we investigated the regulation of release probability in the two nuclei. We measured the time course of the blockade of the NMDA-receptor mediated EPSC with MK801, an activity-dependent open pore blocker, as an proxy for relative release probability. In NA, application of 20-40 μM MK801 blocked over 90% of the NMDAR-EPSC with a wide range of time courses in different neurons (8.6 ± 3.2 trials, n=24). The rate MK801 blockade was slower and more varied in NA versus NM neurons, suggesting release probability is higher on average in NM versus NA. The time course of MK801 blockade and the paired pulse ratio (PPR, 50 Hz) measured in the same NA neurons showed a strong correlation, indicating each is an independent measure related to initial release probability (n=16, r^2 = 0.56).Finally, an analysis of PPR elicited from different sets of fibers onto single NA neurons showed a high correlation across inputs. These results suggest that release probability is regulated in a target-specific manner at auditory nerve terminals: differing across target nuclei but also coordinated across different inputs onto individual target neurons.

Disclosures: J. Ahn: None. **K.M. MacLeod:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.04/PP17

Topic: D.02. Auditory

Support: NIH Grant R03 DC 11361

LA Regents RCS Grant RD-A-09

SVM-CORP USDA 1433 Grant LAV 3300

Title: Functional topography of intrinsic and commissural pathways in the auditory midbrain

Authors: *C. C. LEE[1], Y. YANAGAWA[2], K. IMAIZUMI[1];
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Abstract: The inferior colliculus (IC) is a crucial hub for auditory information processing and integrates inputs from brainstem, midbrain and cortical sources, among others. Yet, although previous anatomical and physiological studies have identified the broad origins and synaptic properties of many of these inputs, the functional topography of these inputs to the IC remains ill defined. There are proportionally high convergent inputs from intrinsic and contralateral IC sources to each of the separate IC subdivisions, the tonotopically organized central nucleus (ICc) and the non-tonotopic shell nuclei: dorsal cortex, external nucleus, and lateral nucleus. Since the IC has unique and distinct roles in auditory processing and behavior, we sought to examine the functional organization of its intrinsic and contralateral inputs. We developed an in vitro slice preparation that maintains intrinsic and commissural connectivity between both colliculi in the mouse. We then assessed the anatomical organization of projections in the slice using anterograde tracing with DiI, and further examined the functional topography of projections using flavoprotein autofluorescence imaging and whole-cell patch clamp recorded responses to laser-scanning photostimulation via uncaging of glutamate. In addition, in order to target inhibitory interneurons for recordings, we utilized a transgenic mouse line with VGAT positive interneurons expressing the Venus fluorescent protein (developed by Atsushi Miyawaki at RIKEN, Wako, Japan). We found that the intrinsic and contralateral projections form intact connections in our slice preparation and that the distribution of inhibitory neurons varies on a nuclear basis. In addition, we observed that the ICc is weakly activated by intrinsic and contralateral electrical stimulation, compared with the surrounding subdivisions. Some IC neurons receive functionally anisotropic intrinsic inputs and topographically segregated excitatory and inhibitory inputs from the contralateral IC, while others displayed opposing patterns. These distinct functional circuits may account for the varied physiological properties observed in vivo and support the diverse IC roles in audition.

Disclosures: C.C. Lee: None. **Y. Yanagawa:** None. **K. Imaizumi:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.05/PP18

Topic: D.02. Auditory

Support: NIH NIDCD R03-DC011589

NIH NIDA T32-DA022616

University of Minnesota Institute for Engineering in Medicine Walter Barnes Lange Memorial Award

University of Minnesota Frieda Martha Kunze Fellowship

University of Minnesota start-up funds

Title: Immediate and residual suppression of neural firing in the inferior colliculus induced by focal electrical stimulation of auditory cortex

Authors: *C. D. MARKOVITZ[1], P. S. HOGAN[1], K. A. WESEN[1], H. H. LIM[1,2,3];
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Abstract: Cortical stimulation has been implicated as a treatment option for several neurological disorders such as stroke, Parkinson's disease, epilepsy, and tinnitus. However, the mechanism of action for cortical stimulation is currently unclear, and little is known about how stimulation can affect neural firing in subcortical structures that are influenced by the cortex. We sought to systematically determine the neurophysiological effect of focal electrical stimulation of the auditory cortex (AC) on the ascending auditory pathway. Particularly, we assessed whether stimulation of the AC could alter acoustic-driven neural firing within the central nucleus of the inferior colliculus (CNIC), the main auditory processing center in the midbrain and a major convergence point for ascending and descending auditory pathways.
We positioned penetrating multi-site electrode arrays into AC and the CNIC in 16 ketamine-anesthetized guinea pigs. After characterizing the acoustic-driven responses on each site to confirm its location (i.e., frequency region and sub-nuclei of AC or CNIC), we electrically stimulated the deeper output layers of AC and recorded the corresponding changes in acoustic-driven responses throughout the CNIC. Additionally, acoustic-driven responses were recorded after electrical stimulation had ceased to determine if residual changes could be induced.
Stimulation of AC output layers induced widespread suppression of acoustic-driven activity throughout the CNIC. The amount of suppression varied drastically depending on the cortical region being stimulated. For example, stimulation of the medial portion of primary auditory cortex induced suppression of nearly half of all the sites in the CNIC, while stimulation of the ventrorostral belt elicited minimal suppression. The suppression was widespread throughout the CNIC without any apparent topographic organization across the tonotopic axis nor along the isofrequency laminae. Furthermore, suppression of neural firing largely continued even after the electrical stimulation had ended. On the other hand, minimal excitation could be elicited anywhere in the CNIC. These results indicate that cortical stimulation can have a profound and lasting suppressive effect on subcortical processing.

Disclosures: C.D. Markovitz: None. **P.S. Hogan:** None. **K.A. Wesen:** None. **H.H. Lim:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.06/PP19

Topic: D.02. Auditory

Title: *In vivo* optical recording of auditory responses in mouse's inferior colliculus using a micro-endoscope

Authors: *H. YASHIRO[1], I. NAKAHARA[4,5], K. I. KOBAYASI[2,3], K. FUNABIKI[4,5], H. RIQUIMAROUX[1,2,3];
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Abstract: The inferior colliculus (IC) is the largest auditory afferent station located in the midbrain. The IC also receives efferent projections from higher brain area. It has been reported that the IC conducts complicated information processing. To understand the neuronal mechanisms underlying its processing, simultaneous recording of multiple neurons is, thus, important. We, therefore, started *in vivo* imaging of mouse's IC using a micro-endoscope. Our endoscope probe consists of thousands of optical fibers (3,000-6,000, Fujikura). The tip of the fiber bundle was beveled as pencil-like shape for brain insertion with minimum invasion. We coated the tip surrounds with Au, and further insulated with enamel paint to use our endoscope as a stimulating and/or recording electrode. The other edge end was polished to have a flat surface and scanned by the galvano mirror based laser scanner. We used resonance galvo mirror (CRS 4 kHz, GSI lumonics) to have fast frame rate (18-100 fps). The field of view was 215 to 300 μm in diameter. The distance between fibers, that determines spatial resolution of the endoscopic image was around 3 μm, enough for discriminating each cell body of the neurons. First, we recorded auditory responses of mouse's IC using a glass capillary containing calcium-sensitive fluorescent indicator (Oregon Green BAPTA A-1, Invitrogen, 800 μM/L). A craniotomy was performed at the position of 1.0 mm dorsal and 1.0 mm lateral to the lambda on a skull. We recorded local field potentials (LFPs) and occasionally single unit activities of the IC neurons against auditory stimuli. Sound stimulus was white noise burst with 50ms duration. The sound pressure level varied from 15 to 95 dB SPL (re: 20 μPa) in steps of 10 dB. Sound stimuli were randomly delivered from a ribbon tweeter (PR-R7III, Pioneer) placed 25 cm in front of the mouse. After characterizing LFP of each location in the IC, 1 μL Oregon green was injected by pressure. These procedures were repeated at the depth of 250, 650 and 1,050 μm from the brain surface 30 min. after dye loading. We inserted our endoscope into the same hole where the glass microelectrode was inserted. Applying the sound stimuli, the LFPs and the images of the IC were

recorded through the endoscope tip at the place where Oregon green was injected. The parameters of the sound stimuli were the same as LFP recordings. We could observe cell bodies labeled with Oregon green and sometimes could observe optical responses following LFP. Cell by cell analysis was sometimes possible. Above preliminary results suggest that our methods can record auditory responses of multiple neurons with cellular resolutions and will be useful in uncovering functional networks in the IC.

Disclosures: H. Yashiro: None. **I. Nakahara:** None. **K.I. Kobayasi:** None. **K. Funabiki:** None. **H. Riquimaroux:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.07/PP20

Topic: D.02. Auditory

Support: NIH Grant DC005787-01A1

Alexander von Humboldt-Foundation

Title: Membrane potential dynamics and spiking correlations in the auditory cortex during spontaneous and tone-evoked activity *In vivo*

Authors: *M. GRAUPNER, A. D. REYES;
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Abstract: Traditionally, auditory cortex is postulated to be organized tonopically such that the characteristic frequencies of individual neurons change continuously along the rostro-caudal axis (Stiebler et al. 1997; Hackett et al. 2011; Guo et al. 2012). However, recent studies with single neuron resolution suggest that the representation of frequency along the tonotopic axis may not be continuous, depending on the cortical layer: the frequency tuning of layer 2/3 neurons tend to be heterogeneous or "salt-and-pepper" (Bandyopadhyay et al. 2010; Rothshild et al. 2010) while those of L3/4 neurons seemed to be continuous (Windowski and Kanold 2013). Furthermore, different acoustic stimuli activate discrete and spatially segregated cell assemblies, further challenging the concept of a spatially continuous mapping (Batherllier et al. 2012). These findings suggest that the functional organization and the underlying network architecture are more complex than previously thought.
Here, we examine the functional connections between neurons and common synaptic drive from shared inputs. We perform simultaneous whole-cell and cell-attached recordings from pairs of

cells in the auditory cortex of ketamine/xylazine-anethetized mice in vivo (P30-45). We record the spiking activity in one cell and the subthreshold membrane potential in the other during spontaneous and tone-evoked activity. Using tone-triggered and spike-triggered average analysis, we examine the existence of a synaptic connection between the two recorded neurons and the amount of common synaptic inputs.
Preliminary results suggest that during spontaneous activity epochs, spatially distant neurons (>500 μm) tend to receive common spontaneous input even though their best frequencies differ substantially. Furthermore, during tone evoked activity, the magnitude of the common input (presumably from thalamus) decrease with increasing difference in best frequency. Together, these results suggest a tightly connected network which maintains a functional segregation for tone representation, possibly emanating from targeted thalamic drive. Combining the analysis of spiking activity and subthreshold activity on a single neuron basis advances our understanding of the interplay between the functional map and the actual connectivity in the auditory cortex and thereby helps to uncover the cortical organization of acoustic representation.

Disclosures: M. Graupner: None. **A.D. Reyes:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.08/PP21

Topic: D.02. Auditory

Support: NIH grant (DC005787-01A1)

Title: Bijective maps between acoustic and cortical Spaces: Representation of tone frequency and intensity in auditory cortex

Authors: *A. D. REYES;
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Abstract: The characteristic frequencies (CF) of neurons vary systematically along the rostro-caudal axis of auditory cortex. This tonotopic organization potentially provides a place code whereby the relative location of the activated neuron contains information about the frequency and intensity of a stimulus tone. However, the main predictions of tonotopy-based coding seem to be inconsistent with recent experiments and network simulations: the gradient in the CFs is imprecise and even a moderate intensity tone is likely to activate many neurons spaced over a large area. Thus, extracting information about tone stimuli using a purely place code is difficult. Here, we provide an alternative representation of tone frequency and intensity in auditory cortex.

Adhering strictly to the mathematical definition of maps and the limitations imposed by the anatomy and by the basic properties of neurons, we derive bijective (one-to-one, onto and hence invertible) maps from the acoustic space to cortical space. To obtain mathematically and biologically well-defined mappings, frequency must be represented by groups of neurons within a continuous area of cortex. The spatial extent of activated neurons increase with tone intensity. To maintain high resolution, there must be substantial spatial overlaps between adjacent groups representing different frequencies; individual neurons can be members of several groups. Indeed, classical cortical columns, which segregate neurons based on receptive field, will increase discontinuities and decrease the resolution. This model, which was derived using mathematical principles and a minimum of well-established neuronal properties, is general and may apply to other cortical areas that use topography to represent sensory input.

Disclosures: A.D. Reyes: None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.09/PP22

Topic: D.02. Auditory

Support: NIH R01DC008983

David and Lucile Packard Foundation

Title: Functional properties of VIP inhibitory neurons in the mouse primary auditory cortex

Authors: *L. MESIK[1], H. W. TAO[2], L. ZHANG[2];
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Abstract: Detailed mapping of local neuronal circuitry will require a good understanding of its many underlying components. The apparent diversity of inhibitory neurons poses a particular challenge as these neurons are small, relatively few, and hard to target in vivo. In recent years, most attention has been paid to the two largest subgroups of these interneurons, those expressing parvalbumin(PV) and somatostatin(SOM). Other subclasses, such as the interneurons expressing the neuropeptide VIP (vasoactive intestinal peptide) remain less well studied. We applied two-photon imaging guided patch-clamp recording to characterize the functional responses of VIP interneurons in layer 2/3 of the mouse primary auditory cortex. Compared to PV neurons, VIP neurons respond relatively slowly, often integrating input over 10 or more milliseconds before spiking. However, their response is also more reliable than that of the similarly sluggish SOM

neurons. Overall, our results imply that the VIP neurons fulfill a functional role distinct from that of the other major inhibitory subgroups.

Disclosures: L. Mesik: None. **L. Zhang:** None. **H.W. Tao:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.10/PP23

Topic: D.02. Auditory

Support: Funding Program for Next Gneneration World-Leading Researchers (NEXT program)

Title: Multielectrode array recording of propagation of activity evoked by electrical micro-stimulation in horizontal and coronal slices of the mouse auditory cortex

Authors: *H. KITAMURA, J. NISHIKAWA, T. TATENO;
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Abstract: In the auditory cortex of mice, little is known about how propagating activity is generated by sound signals and where it propagates. Recent studies indicated that recording from in vitro brain slices with micro electrical stimulation gave some insight into in vivo propagating activity of the auditory cortex.
In this study, using multielectrode array (MEA) substrates, we recorded electrically evoked neural activity from horizontal and coronal slices of the mouse auditory cortex. After in vivo optical imaging by use of voltage-sensitive dyes in response to sounds, some fields in the auditory cortex were first identified, slices were then prepared and multielectrode recording was afterward carried out. We performed electrical stimulation at both shallow (II/III) and deep (V/VI) layers of the auditory cortex on electrodes on the MEA substrate, and recorded local field potentials (LFP), whose main components were likely to be EPSPs, IPSPs, and/or population spikes.
In the experiments, stimulation of a short current pulse (0.03 - 0.1 mA) evoked activity of LFP (0.05 - 1.0 mV) in proportion to the amplitude of the current. Firstly, after stimulating at deep layers, the activity evoked from the deep layers propagated to shallow layers and then propagated through the surrounding shallow layers. Moreover, results of evoked propagations through the shallow layers in horizontal slices on the MEA had some similarities in propagating directions as those obtained from optical imaging by voltage-sensitive dyes. Secondly, to separate the LFP signals into several components mediated by specific receptors, we applied several blockers to extracellular solutions. Thus, we found that a main component of the LFP

signals was likely to be AMPA receptor mediated currents, although NMDA receptor and other ion channels also had some contributions.
We are now developing artificial devises with a MEA and an acoustic sensor, which can be directly implanted in the auditory cortex. These results have a possibility to understand where appropriate sites are to evoke auditory neural activity and which temporal stimulation signals are most effective to evoke the activity in the auditory cortex.

Disclosures: H. Kitamura: None. **J. Nishikawa:** None. **T. Tateno:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.11/PP24

Topic: D.02. Auditory

Support: NIH DCD

Title: Intracortical multiplication of thalamocortical signals in mouse auditory cortex

Authors: *L.-Y. LI[1], Y.-T. LI[1], L. A. IBRAHIM[1], H. W. TAO[1], L. I. ZHANG[2];
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Abstract: Cortical processing of sensory information initiates from the transformation of thalamically-relayed signals. In thalamorecipient layers of sensory cortices, the thalamic signals have been thought to be augmented and expanded by intracortical excitatory inputs. To determine the precise functional role of intracortical excitation in thalamocortical transformation, we silenced intracortical excitatory circuits in a reversible manner by optogenetically activating cortical parvalbumin positive inhibitory neurons. In vivo whole-cell voltage-clamp recordings were carried out in layer 4 of mouse primary auditory cortex to compare the isolated thalamocortical input with the total excitatory input evoked by auditory stimuli. We found that intracortical excitatory circuits preserved the frequency and direction tuning of thalamocortical input by linearly amplifying the thalamocortical signals while prolonging the response duration. There was no change in the spectral range of excitatory responses after cortical silencing, suggesting a lack of recruitment of distant inputs through horizontal circuits. The input-specific preamplification and the response prolongation by intracortical excitation will largely enhance the salience of afferent signals, and may ensure a robust, faithful and more persistent representation of thalamocortically relayed information.

Disclosures: L. Li: None. **Y. Li:** None. **L.A. Ibrahim:** None. **H.W. Tao:** None. **L.I. Zhang:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.12/QQ1

Topic: D.02. Auditory

Support: NIH Grant DC006013

Department of Anesthesiology

School of Medicine and Public Health

Title: Spatiotemporal activity patterns in auditory cortex deviate from those predicted by the canonical microcircuit model

Authors: *B. M. KRAUSE[1,2], A. RAZ[2,4,5], D. J. UHLRICH[3], P. H. SMITH[3], M. I. BANKS[2,3]; [1]Neurosci. Training Program, [2]Dept. of Anesthesiol., [3]Dept. of Neurosci., Univ. of Wisconsin, Madison, WI; [4]Dept. of Anesthesiol., Rabin Med. Ctr., Petah-Tikva, Israel; [5]Dept. of Anesthesiol. and Critical Care Med., Sackler Sch. of Medicine, Tel Aviv Univ., Tel Aviv, Israel

Abstract: Introduction: Cortical columns integrate extrinsic and intrinsic afferent input to generate spatio-temporal activity patterns. This process is of fundamental importance for understanding the neural basis of perception and cognition. The canonical microcircuit model derived from studies of visual and somatosensory cortex posits that the cortical laminae are activated in a L4 to L2/3 to L5 sequence in response to core thalamocortical (TC) input. Matrix thalamocortical and descending corticocortical inputs terminating in layer 1 (L1) and infragranular layers are postulated to modulate this activation sequence. However, the applicability of this model to auditory cortex (A1) is unclear, as cell types and TC afferent termination patterns differ in A1 from other cortical areas.
Methods: We investigated the laminar profile of synaptic and spiking responses to TC, L1 and paired afferent stimulation in auditory TC slices prepared from 4-10 wk old male mice (B6CBAF1/J). Approximately whole-column (800x200 m) Ca imaging (OGB-1 AM) was used to identify spiking cells as a function of laminar depth. Synaptic and spiking responses on finer time scale were measured using patch clamp recordings from cells in layers 2 - 6.
Results: Auditory TC slices were observed to dwell almost exclusively in DOWN states, with rare spontaneous UP states but reliable evoked UP states in response to afferent stimulation.

Contrary to the predictions of the canonical microcircuit model, cells throughout the column receive TC synaptic input at near equal latency, and spiking in auditory cortical columns is dominated by cells in L5. Monosynaptic spiking is sparse (<5% at moderate stimulus intensity) and disynaptic spiking was not observed except in the context of UP state activity. The majority of cells that spike monosynaptically are interneurons, especially L5 Martinotti cells that project to L1. This ascending activation pattern is only weakly suppressed by L1 input.
Conclusions: These data suggest that the integrative properties of the cortical column may be altered substantially in DOWN states, and that modifications of the canonical microcircuit model may be required for columns in auditory cortex.

Disclosures: B.M. Krause: None. **A. Raz:** None. **D.J. Uhlrich:** None. **P.H. Smith:** None. **M.I. Banks:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.13/QQ2

Topic: D.02. Auditory

Support: 1R21-NS079929

Title: A circuit basis for motor cortical modulation of auditory cortical activity

Authors: *A. NELSON, D. SCHNEIDER, J. TAKATO, F. WANG, R. MOONEY;
Duke Univ., Durham, NC

Abstract: Animals must distinguish sensations arising from their own movements from sensations arising from objects in their environment. This can be accomplished in part by transmitting a copy of the motor plan to relevant sensory systems. In the context of hearing, motor-related signals are thought to impinge on many levels of the auditory system, including in the cortex. Dysfunction of intracortical motor-auditory circuitry is believed to underlie symptoms of debilitating neuropsychiatric disorders, including the auditory hallucinations characteristic of schizophrenia and other forms of psychoses. Despite the importance of intracortical motor-auditory interactions in normal and diseased states, little is known about the synaptic and circuit mechanisms underlying this interaction. We performed a series of experiments in the mouse to detail the architecture and function of a specific population of motor cortical neurons that provide input to the auditory cortex and thus are well poised to transmit motor-related signals to the auditory cortex. Using dual retrograde tracing and intersectional viral anterograde tracing, we found that a subset of neurons in the medial agranular motor cortex that project to auditory

cortex and also project to subcortical regions, including periaqueductal gray (PAG), pons, and brainstem, highlighting a circuit capable of relaying copies of descending motor commands to the auditory cortex. Using in vitro whole cell physiology, optogenetics, and pharmacology, we find that motor cortical axons make excitatory synapses in the auditory cortex but exert a net inhibitory effect on both superficial and deep layer auditory cortical neurons through feedforward inhibition mediated in part by PV+ auditory cortical interneurons. Finally, using in vivo intracellular physiology, optogenetics, and sound playback, we show that directly activating motor cortical axon terminals in the auditory cortex suppresses spontaneous, tone-evoked, and naturalistic stimuli-evoked synaptic activity in auditory cortical neurons, and that this effect is dependent on the relative timing of motor cortical activity and auditory stimulation.

Disclosures: A. Nelson: None. **D. Schneider:** None. **J. Takato:** None. **F. Wang:** None. **R. Mooney:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.14/QQ3

Topic: D.02. Auditory

Support: RIKEN Brain Science Institute

Medical Research Council (MR/J004448/1)

Tenovus Scotland (S11/1)

Deafness Research UK (552:STR:SS)

Title: Interlaminar processing in auditory cortex: Spontaneous and evoked responses of independent sources

Authors: *E. MUNRO[1], T. KHODAI[2], S. SAKATA[2], T. TOYOIZUMI[1];
[1]RIKEN Brain Sci. Inst. - Wako, Saitama, Japan; [2]Strathclyde Inst. of Pharm. and Biomed. Sci., Univ. of Strathclyde, Glasgow, United Kingdom

Abstract: The cortical local field potential (LFP) reflects synchronous activity of populations of neurons at various frequencies ranging up to 600 Hz. LFP signals have been successfully used to predict sensory stimuli or an animal's behavioral state. Although the LFP signal is known to be a summation of multiple neural sources, most conventional studies analyze it by looking at the current source density or blindly combing several LFP signals for decoding. In this study, we

investigate interlaminar processing in auditory cortex by applying independent component analysis (ICA) to recorded LFP signals, namely, by decomposing LFP signals into independent neural sources. Rats were anesthetized with urethane anesthesia, and recordings were taken from a 16 or 32 channel silicon probe inserted vertically into the auditory cortex. To compare normal neocortical processing with processing after trauma, we applied auditory trauma which consisted of 110 dB white noise played for one hour. We recorded spontaneous activity and reactions to short tones and long tones of various frequencies and intensities before and after the trauma. We applied ICA to data recorded during spontaneous activity, short tones, and long tones separately. ICA separates temporally independent neural sources and gives a spatial profile of how the time series of each source is projected onto the recording electrodes. From these spatial profiles, we are able to estimate the size and location of each neural source. ICA identified many sources from each layer. These sources include: a far away or wide-spread source producing a global slow oscillation, a layer 4 source that robustly responds to tones, and a strong layer 5 source. Average tone responses reveals multiple neural sources with similar spatial profiles that act distinctly to different tones and intensities, suggesting the existence of multiple groups of neurons in the same layers that uniquely code auditory information.

Disclosures: E. Munro: None. **T. Khodai:** None. **S. Sakata:** None. **T. Toyoizumi:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.15/QQ4

Topic: D.02. Auditory

Support: NIH DC-011874

NIH DC-02260

Coleman Memorial Fund

Hearing Research, Inc.

Title: Auditory cortical local subnetworks are characterized by sharply synchronous activity

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Abstract: In primary auditory cortex (AI), broadly correlated firing has been commonly observed. In contrast, sharply synchronous firing has been rarely seen and its properties and

functional consequences are not well understood. Therefore, we examined AI local subnetworks using cross-correlation analysis and spectrotemporal receptive fields (STRFs). Sharply synchronous firing responses were independent of layers and were present between all distinguishable cell-types. The sharpest synchrony was seen in supragranular layers and between regular spiking units. Synchronous spikes conveyed more stimulus information than non-synchronous spikes. Neighboring neurons in all layers had similar best frequencies (BFs) and similar STRFs, with the highest similarity in supragranular and granular layers. Spectral tuning selectivity and latency were only moderately conserved in these local, high-synchrony AI subnetworks. Overall, sharp synchrony is a specific characteristic of fine-scale networks within AI, and local functional processing is well-ordered and similar, but not identical, for neighboring neurons of all cell types.

Disclosures: C.A. Atencio: None. **C.E. Schreiner:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.16/QQ5

Topic: D.02. Auditory

Support: NICHD Intramural Program

Title: Gsx1 specified neurons are required for prepulse inhibition

Authors: *H. A. BURGESS[1], G. LI[1], N. CARRIER[2], S. A. BERGERON[1];
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Abstract: Prepulse inhibition (PPI) of the startle response is a widely used paradigm for studying sensorimotor gating. During PPI a weak stimulus suppresses the magnitude of the startle response to an intense stimulus. Reduced PPI has been reported in neurological conditions including schizophrenia, highlighting the need to understand the basic neuronal circuitry that mediates this behavior. Previous work has shown that PPI is present in fish and has several characteristic features of mammalian PPI. To identify neuronal components of the PPI pathway we carried out a circuit breaking screen in larval zebrafish. We first performed a Gal4 enhancer trap, including neuronal restrictive silencing elements in the vector to enrich for lines with brain specific expression. We then genetically ablated trapped neurons in brain specific lines using a UAS:Nitroreductase transgene and tested larvae for PPI. After ablation, line *y252* showed defects in startle sensitivity and PPI that were strongly dependent on the interstimulus interval. Defects

in startle regulation were reproduced by acute optogenetic inhibition of the trapped neurons with UAS:Archaerhodopsin3 demonstrating that the defect is not secondary to a disruption in neural development. The most salient expression of Gal4 in *y252* is in a bilateral, dorsally positioned, longitudinal column of glutamatergic neurons in the hindbrain. These neurons are commissural and have neurites extending close to the Mauthner cell, the command neuron for the startle response. Using a UAS:Synaptophysin-RFP reporter, we found synapses from *y252* neurons in close apposition to the lateral dendrite of the Mauthner cell. We mapped the transgene insertion to a region close to the *gsx1* gene. *gsx1* shows transient expression in hindbrain neurons which is almost identical to *y252* Gal4 expression demonstrating that *gsx1* specifies neurons labeled in this line. The reported pattern of *Gsx1* expression in mouse closely resembles that in zebrafish, leading us to test *Gsx1* knockout mice for PPI. We found a robust attenuation of PPI in homozygous mutant pups, arguing that *Gsx1* neurons play a similar role in the PPI circuit in mammals. Together, these findings provide strong evidence that neurons required for PPI are glutamatergic commissural interneurons which likely make direct connections with startle command neurons and are conserved across vertebrates.

Disclosures: H.A. Burgess: None. **S.A. Bergeron:** None. **G. Li:** None. **N. Carrier:** None.

Poster

548. **Auditory System: Synapses, Circuits, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 548.17/QQ6

Topic: D.02. Auditory

Support: IOS 0946637

IOS 11471172

Title: Inhibitory components of synaptic PPI in the goldfish auditory startle circuit

Authors: *P. CURTIN[1], T. PREUSS[2];
[1]Biopsychology, City Univ. of New York, Hunter Col., New York, NY; [2]Psychology, Hunter College, CUNY, New York, NY

Abstract: Prepulse inhibition (PPI) is a sensorimotor gating process that reduces the excitability of startle responses when weak, non-startling stimuli are presented 20 -500 ms prior to startling stimuli. The Mauthner-cell (M-cell), the decision-making neuron of the teleost startle circuit, presents an ideal preparation for studying prepulse inhibition because it constitutes the final common path for tonic and phasic inhibitory mechanisms that contribute to PPI. Here we applied

in vivo electrophysiology and pharmacology in goldfish (*Carassius auratus*) to determine what inhibitory neurotransmitters produce PPI at the level of the M-cell circuit. The M-cell is extensively innervated by glycinergic and GABA-ergic projections from associated inhibitory networks. Accordingly, we applied strychnine to the brain to test the effects of systemic antagonism of glycine receptors on tonic and phasic inhibition of the Mauthner cell. The magnitude and time-course of PPI were quantified by presenting two auditory stimuli (prepulse and pulse) at inter-stimulus intervals (ISIs) ranging from 20 - 500 ms, and measuring the attenuation of M-cell sound-evoked post-synaptic potentials (PSPs) produced by prepulses. As expected, blockage of glycine receptors enhanced the intrinsic excitability of the M-cell, profoundly changing the waveform of the sound-response. These effects resulted in a significant enhancement of PSP amplitude and an increased latency to the peak of the sound response. Despite these changes in tonic excitability, the time-course and magnitude of PPI remained robust at all ISIs exceeding 50 ms. Strikingly, PPI at the shortest ISI tested, 20 ms, was largely abolished by strychnine administration, suggesting that the inhibition produced at this ISI is distinct from others, likely reflecting the contribution of the well-characterized feed-forward inhibitory system in the auditory startle circuit. These results suggest that the neurotransmitter mechanisms underlying PPI at the synaptic level are not glycinergic; further, the circuits that produce PPI are likely not mediated by the action of glycine receptors. Ongoing experiments focus on characterizing GABA-ergic components of PPI with local and systemic applications of the GABA antagonists.

Disclosures: P. Curtin: None. **T. Preuss:** None.**Poster**

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.01/QQ7

Topic: D.02. Auditory

Support: NHMRC grant APP630618

Title: Mouse gene expression analysis following temporary threshold shift

Authors: *J. M. CEDERHOLM[1], K. E. FROUD[1], A. F. RYAN[2,3], G. D. HOUSLEY[1]; [1]Dept. of Physiol., Univ. of New South Wales, Sydney, Australia; [2]Surgery and Neurosciences, Univ. of California at San Diego, La Jolla, CA; [3]San Diego Veterans Admin. Med. Ctr., La Jolla, CA

Abstract: Acoustic overstimulation, and the associated hearing loss, is an increasing global problem in today's society, including occupational exposure (e.g. military, industrial) and an

increasing prevalence of sustained recreational exposure with the use of personal music players. Moderate noise exposure leads to a temporary threshold shift (TTS) of hearing sensitivity, reversible within hours to days, while higher noise exposure leads to a permanent threshold shift (PTS) either immediately, or by delayed impact evident with aging. Many cochlear structures are known to be affected by noise overstimulation, and changes in gene expression after PTS-inducing noise have also been identified. However, transcriptional responses underlying TTS, and likely reflecting physiological responses to acoustic stress, have not been investigated. We used a gene array approach to determine cochlear gene regulation in response to TTS-inducing noise in C57Bl/6J mice. Mice were anaesthetised with a ketamine/xylazine/acepromazine cocktail in accordance with University of New South Wales' Animal Care and Ethics Committee approval. Evoked auditory brainstem responses (ABR) were measured to broadband clicks, or 16 kHz tonepips, before and after the noise exposure (86 dB or 95 dB, 4 - 32 kHz, 30 min), to quantify the TTS. Cochlear RNA extraction was performed 1, 2, 4, 8 or 24 hrs after noise exposure. cDNA templates were hybridised to the Affymetrix® mouse gene array 1.1ST, with gene expression analysis performed using GenePattern software. The level of TTS following 86 dB noise was ~ 12 dB, which recovered within 96 hrs. Threshold shifts of ~ 40 dB were produced with the 95 dB noise, recovering within two weeks. 63 genes were significantly regulated compared to no-noise controls ($p<0.001$; $q<0.1$; minimum 2-fold change), with the largest number of responsive genes at 4 hrs. 18 of the genes found in the 86 dB study, were also found at 95 dB. The genes identified were mainly involved in transcriptional regulation, tight junction, morphology, cell migration, inflammatory and oxidative stress responses. We have shown, for the first time, transcriptional responses to TTS-inducing noise. The TTS-regulated gene set we have identified likely reflect cellular responses to noise stress that contribute not only to loss of sensitivity, but also to physiological hearing adaptation and protection from noise-induced hearing loss.

Disclosures: J.M. Cederholm: None. **K.E. Froud:** None. **A.F. Ryan:** None. **G.D. Housley:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.02/QQ8

Topic: D.02. Auditory

Title: Categorical perception of communication sounds and a learned category through sound discrimination training revealed by mismatch negativity in Mongolian gerbils

Authors: *Y. TORIGOE[1], K. I. KOBAYASI[2,3], H. RIQUIMAROUX[1,2,3];
[1]Grad. Sch. of Life and Med. Sci., [2]Biomed. Information, Fac. of Life and Med. Sci.,
[3]Neurosensing and Bionavigation Res. Ctr., Doshisha Univ., Kyoto, Japan

Abstract: Mongolian gerbils communicate with each other by vocalization. This study recorded mismatch negativity (MMN) in the auditory evoked potential (AEP) for measuring the categorical perception of communication sounds and the acquirement of an auditory category. Five animals were implanted an epidural electrode for measuring AEP. We synthesized stimuli mimicking "greeting call", which was produced by both males and females in friendly contact. A typical greeting call is an upward frequency modulated sound (upFMs) where the frequency sweeps from 29 to 34 kHz with duration of ca. 34 ms. About 80% of the call were observed between 27 to 37 kHz; therefore, a stimulus within the range was defined as "within" the greeting call range, and stimulus below or above the range were defined as "outside" of it.
In experiment 1; we used eight types of stimulus. Four types were upFMs. Their frequency swept in different range: 27-31 kHz (within), 32-37 kHz (within), 22-26 kHz (outside) and 38-43 kHz (outside). Four types downward (down) FMs were temporally reversed upFMs; all downFMs were regarded as "non-communication (non-cmm)" sounds. Durations of all stimuli were 34 ms. MMNs were recorded from awake animals when they were passively listening to a series of FMs played with standard oddball paradigms. MMN to upFMs elicited by across-boundary pair (change "within" to "outside") was larger than within pairs. MMNs to downFMs responding to across-boundary pair was not different from within pairs.
In experiment 2; we trained gerbils to discriminate FMs by Go/NoGo task with electric shocks. Both upFMs 32-37 kHz (within) and downFMs 37-32 kHz (non-cmm) were used as Go stimuli, while both upFMs 22-26 kHz (outside) and downFMs 26-22 kHz (non-cmm) were used as NoGo stimuli. After the training was completed, behavioral responses toward FMs between Go and NoGo stimulus were measured. The categorical boundary learned by the training was estimated as between 24-28 kHz and 27-31 kHz FMs for both up and downFMs. MMNs were recorded before and after training. MMN to upFMs across the boundary were significantly larger than within the boundary regardless of the training. The result was accordance with exp. 1, because the obtained boundary was roughly matching the lower limit of communication sounds. While, downFM pair across the obtained boundary elicited significantly larger MMNs than the pair within the boundary only after the training. In all, our study demonstrated that (1) differences in MMNs revealed that acoustic characteristics (frequency range and types of FM) of communication sound were encoded in gerbils' auditory system and (2) the MMN differences can be obtained through behavioral learning.

Disclosures: Y. Torigoe: None. **K.I. Kobayasi:** None. **H. Riquimaroux:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.03/QQ9

Topic: D.02. Auditory

Title: Decrement in cochlear microphonics to a repeated sound in Mongolian Gerbil, Meriones unguiculatus

Authors: *S. BOKU[1], K. I. KOBAYASI[2,3], H. RIQUIMAROUX[1,2,3];
[1]Grad. Sch. of Life and Med. Sci., [2]Biomed. Information, Fac. of Life and Meidical Sci.,
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Abstract: Variety of repeated sounds is overflowing in natural soundscapes. The adaptation or habituation to non-significant repeated sounds is fundamentally crucial to survive for many animals. In the auditory system, the stimulus-specific adaptation (SSA), a decrease in neuronal responses to repeated sound stimuli, has been reported at different levels along the central auditory pathway. However, SSA in the auditory periphery has not yet been studied well because there has been an auditory fatigue discussion. The cochlear microphonic (CM) is a useful tool for understanding the auditory periphery. The purpose of the present research was to examine SSA in the CM. Experiments were performed on five adult Mongolian gerbils (Meriones unguiculatus), the typical auditory animal model, with body weights between 75 and 90 g. The right cochlea of each gerbil was implanted a chlorinated silver-wire electrode at the round window to record CM under anesthesia, induced by a mixture injection of ketamine and xylazine. Awake gerbils were comfortably held with harness in a sound attenuated Faraday cage. A calibrated loudspeaker was set at a distance of 10 cm from the right ear. Tone bursts with 50 ms duration and 3 ms rise/fall times of two frequencies (f1: 4 kHz and f2: 2.8 kHz) were used for sound stimuli. Sound stimuli were presented in two paradigms. In a test paradigm, a block consisting of 50 repeated identical tone bursts (f1) followed by 10 repeated identical tone bursts (f2) was presented 15 times. All tone bursts had the same amplitude (70 dB SPL) and repetition rate (3 Hz). The other paradigm for control condition consisted of both frequencies (f1 and f2) with equal probability in pseudo-random order; however, repetitive rate was 6 Hz. Therefore, control paradigm contained the same number of tone bursts in the same time period as test paradigm. The averages of all CM response amplitudes were calculated. As results, we observed a reduction in CM amplitude before 50 presentations (f1) to repetitive test sound stimuli were presented, especially within first 20 presentations. In addition, after 10 repetitions of a different frequency (f2) for disadaptation, CM amplitudes responding to test frequency (f1) recovered. Whereas, in control stimuli, CM amplitudes did not change even though total number of tone bursts (f1) was identical to that for the test paradigm. The data may indicate that the decrement in CM amplitude is not caused by auditory fatigue because CM amplitude will not recover immediately following temporary inner ear impairment. Moreover, the reduction did not depend

on the amount of tone bursts in control. Therefore, the results suggest that SSA occurs in the auditory periphery.

Disclosures: S. Boku: None. **K.I. Kobayasi:** None. **H. Riquimaroux:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.04/QQ10

Topic: D.02. Auditory

Support: NIH R01 DC007690

NIH F31 DC012000

Title: Organized motion direction selectivity mediated by response adaptation in the owl's inferior colliculus

Authors: *Y. WANG, J. L. PENA;
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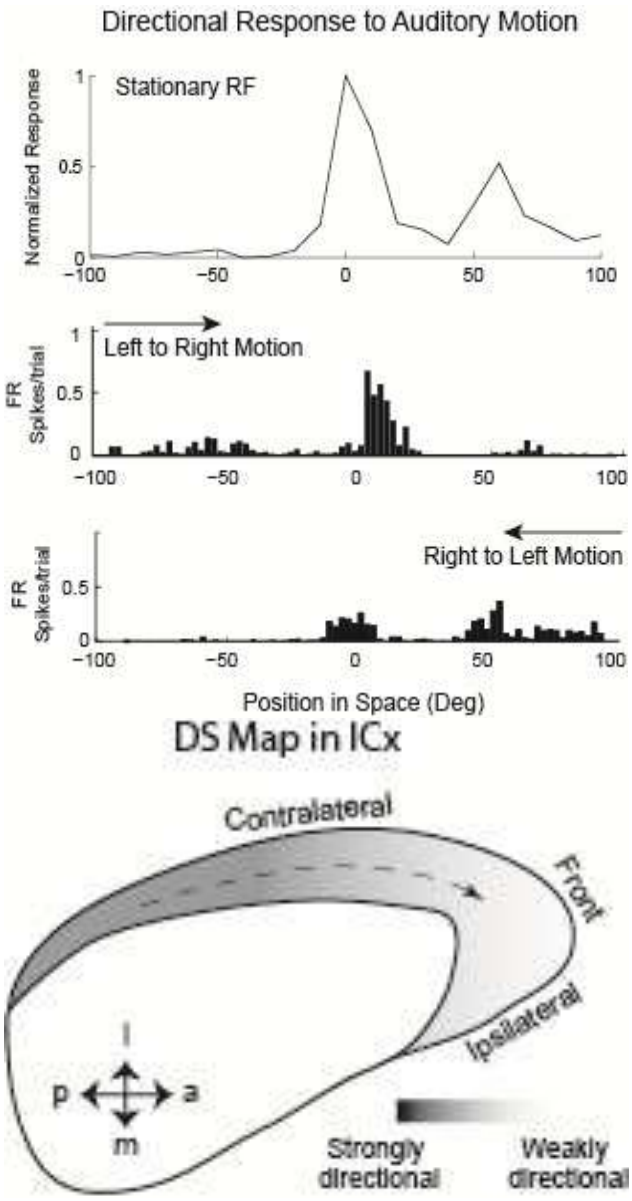
Abstract: Motion-direction selectivity (DS) is a common feature of sensory systems. However, mechanisms that confer DS in auditory space are not well understood. It is unclear whether DS in the auditory system uses similar mechanisms as those proposed in the visual and somatosensory systems or whether DS to motion is represented in an orderly manner in auditory nuclei. In the current study, we investigated these questions in neurons of the owl's external nucleus of the inferior colliculus (ICx) using apparent sound motion in a high density free-field speaker array. We found that preferred direction could be predicted by asymmetries in the spatial receptive field (RF). At the population level, we found a systematic increase in RF feature asymmetry which corresponded to the measured increase in directional preference for sounds moving toward frontal space as a function of eccentricity in spatial tuning. Response adaptation could explain DS observed in the ICx because firing attenuates the response to later stimuli. The strength of the attenuation or adaptation decreased with time between stimuli. A linear model based on spatiotemporal summation using excitation and adaptation parameters estimated from the data accurately predicted the direction-dependent response during sound motion.



Disclosures: Y. Wang: None. **J.L. Pena:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.05/QQ11

Topic: D.02. Auditory

Support: NIH RO1 DC005640

Title: Towards an antibody toolkit for array tomography in avian species

Authors: *K. E. PANNONI[1], A. MUKHTAR[2], D. RYBKA[2], W. DEBELLO[2];
[1]Ctr. for Neurosci., Univ. of California, Davis, CA; [2]Ctr. for Neuroscience, Univ. of California, Davis, CA

Abstract: Array tomography is a method to determine the molecular fingerprint of each synapse within fixed tissue. It relies on antibody detection of synaptic and neuronal proteins. The large majority of commercially available antibodies were raised against immunogens derived from mammalian peptide sequences. Although these protein targets are in large part evolutionarily conserved (with important exceptions, e.g. synapsin), specificity of the antibodies for avian brain tissue must be determined empirically. We performed western blotting on protein homogenates derived from rat, mouse, chicken, barn owl, zebra finch and canary brain. Antibodies that produced expected banding patterns across mammals and birds were used as probes for array tomography. Arrays were made of the barn owl inferior colliculus, a model circuit for information processing, plasticity and learning. Successive imaging of stained, stripped and re-stained arrays produced high-resolution, seven-color image volumes of ~100,000 cubic microns. Antibodies to synaptic proteins SV2c, vGlut1/2, GAD and Homer 1 each produced dense, punctate staining. Punctae are being characterized manually, with object-based classifiers and with feature-based classifiers, to determine if they conform to expectations for synaptic sub-populations. Antibodies against tubulin revealed neuropil very similar to that observed in mammalian tissue. In summary, we have taken the first steps towards vetting of an antibody toolkit that should be widely applicable to avian neuroscience model systems.

Disclosures: K.E. Pannoni: None. **A. Mukhtar:** None. **D. Rybka:** None. **W. DeBello:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.06/QQ12

Topic: D.02. Auditory

Support: R01 DC005640

Title: Neuronal circuits underlying attention-dependent learning in the barn owl

Authors: *D. SANCULI, W. DEBELLO;
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Abstract: Both the neural substrates underlying attention and the synaptic mechanisms underlying learning are beginning to be understood, yet little is known regarding their interface. Recent studies in the barn owl (*Tyto alba)* have shown that top-down allocation of an attention-like process can influence low-level responses to both visual and auditory stimuli. The control structure involved in this sensory modulation is the arcopallial gaze fields (AGF) and its target is the optic tectum (OT). The OT is also known to be the primary source of instructive information that guides adaptation to visually displacing prisms. This learning occurs reliably in juvenile owls even under passive conditions. In contrast, it does not occur in adults unless owls are required to hunt live mice, a highly engaging task. One possibility is that an attentional gate on delivery of instructive information develops during maturation from juvenile to young adult. If true, lesions of the AGF are predicted to halt learning in adult hunters but not in passive juveniles or juvenile hunters. To test this hypothesis, electrophysiological surveys are underway in five prism-adapted juveniles, to confirm reliable shifts in auditory tuning. Chemical lesions will be made in the AGF and the same individuals will be actively challenged as young adults. In addition, a cohort of naïve juveniles and naïve adults, with and without lesions, will be examined.

Disclosures: D. Sanculi: None. **W. DeBello:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.07/QQ13

Topic: D.02. Auditory

Support: R01 DC005640

Title: Functional connectivity within and across structures in the barn owl midbrain in response to auditory and visual stimulation

Authors: *D. TOTTEN, W. DEBELLO;
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Abstract: We are using multielectrode arrays to measure correlations in firing between synaptically connected neurons in the barn owl auditory localization pathway. The targets are feedforward connections from ICCls to ICX and bidirectional connections between the ICX and

dOT. To simultaneously measure paired responses, a 7-channel array (Thomas Recording) with tight concentric spacing was deployed in one structure and a single tungsten electrode in the other. Auditory and visual receptive fields were determined using, respectively, dichotic noise bursts and static, looming or moving dots presented via a DLP projector. The auditory and visual tuning of all sites within the array was similar, and electrode tract reconstruction confirmed sampling of nearby neurons. Functional connectivity was determined using cross-correlation analysis with shuffle. Preliminary results were obtained from 3 owls with sites located in ICX or dOT. Correlograms were diverse in profile, indicating heterogeneous connectivity patterns even among neighboring neurons. Tight peaks were found in a subset of pairs, suggesting neurons with common drivers and/or monosynaptic interconnections. This approach is now being applied to analysis of prism-adapted owls, to probe both the circuit mechanisms that guide learning and the functional consequences of the structural re-wiring that is known to occur. ICCls = lateral shell of the central nucleus of the inferior colliculus. ICX = external nucleus of the inferior colliculus. dOT = deep layers of the optic tectum.

Disclosures: D. Totten: None. **W. DeBello:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.08/QQ14

Topic: D.02. Auditory

Support: CNPQ

FAPEMIG

CAPES

Title: Effects of fear conditioning on auditory steady-state responses in inferior colliculus

Authors: *A. V. LOCKMANN, F. A. G. MOURÃO, M. F. D. MORAES;
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Abstract: In this work, we tested the hypothesis that associative-learning potentiates and modifies the timing of steady-state processing in local circuits of inferior colliculus (IC). Local field potentials were recorded over IC of freely-behaving rats submitted to paired or unpaired auditory fear conditioning. Auditory steady-state responses (ASSR) were elicited by a 10 kHz tone, amplitude modulated at 53.7 Hz, which was used as the conditioned stimulus (CS). Both

amplitude and phase of ASSR showed learning-related changes in paired group, but with inverse temporal dynamics. When animals were re-exposed to CS early on test, phase component consistently shifted from its pre-conditioning values. This initial shift reversed with repeated expositions to CS, and this reversal coincided with an increase in amplitude. These effects were not seen on unpaired group, suggesting that conditioning related changes on ASSR components reflected a modification in the pattern of local processing of CS due to associative-learning.

Disclosures: A.V. Lockmann: None. **F.A.G. Mourão:** None. **M.F.D. Moraes:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.09/QQ15

Topic: D.02. Auditory

Support: Supported by NIH grants DC00115 and DC-05211.

Title: Stimulus specific adaptation is stronger at short latencies in the inferior colliculus

Authors: *T. RUBIN[1], E. D. YOUNG[2];
[1]Biomed. Engin., Johns Hopkins, Baltimore, MD; [2]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Stimulus specific adaptation (SSA) allows auditory neurons to respond more strongly to a stimulus when infrequently presented, relative to the response when frequently presented. Since originally described in cortex (by Ulanovsky and Nelken), SSA has been demonstrated to occur in the inferior colliculus (IC), but not at lower levels. We are investigating SSA in the IC of awake marmosets; the phenomenon can be observed in this system unmodified by anesthesia, allowing late phenomena to be observed in tonic responses. Previously we showed robust SSA in this preparation. The properties of the neurons showing the strongest SSA suggest that they are in the external nucleus or dorsal cortex (collectively ICX) rather than in the central nucleus (ICC). As the ICX receives strong descending input from cortex and thalamus as well as ascending inputs from brainstem nuclei, the SSA in ICX may be produced by either descending inputs. Consistent with this hypothesis, ICX neurons often show complex temporal responses which may outlast a brief (50 ms) auditory stimulus by up to 100 ms. A simple hypothesis might be that short latency responses would reflect ascending inputs and not show SSA whereas long latency responses would reflect descending inputs and show SSA. To test this hypothesis we measured SSA across the driven responses of single neurons in ICC and ICX. We show that the extent of SSA does indeed vary between short and long latency components of responses.

Surprisingly, there is more SSA in short-latency responses (<~50 ms) than in later responses. This SSA correlated with both increasing spike front latencies and adaptation over the course of multiple stimuli. Our results thus suggest that SSA is created de-novo on the IC.

Disclosures: T. Rubin: None. **E.D. Young:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.10/QQ16

Topic: D.02. Auditory

Support: Scientific & Technological Cooperation Programme Switzerland-Russia

Swiss National Science Foundation 31003A_127024

European Union ERC-2010 AdG 268911

Title: Hierarchical network of vocalizations in songbird groups

Authors: *A. L. VYSSOTSKI[1], V. N. ANISIMOV[2], A. V. LATANOV[2], R. H. R. HAHNLOSER[1];
[1]Inst. of Neuroinformatics, Univ. of Zurich and ETH Zurich, Zurich, Switzerland; [2]Fac. of Biology, Moscow State Univ., Moscow, Russian Federation

Abstract: Vocalizations are an essential part of social interactions in birds. They have important biological functions in coordinating activity of group members. However, little is known about the structure of vocal interactions in bird groups. The main challenge for investigating vocal interactions and their biological significance in a bird group is to discriminate individual vocalizations of rapidly moving, sometimes simultaneously vocalizing individuals. We make use of recently developed back-attached ultra-miniature sound/acceleration recorders (SfN 2011, 692.08) and recorded vocalizations in groups of freely moving laboratory-housed zebra finches. Conveniently, the microphone picked up sounds from several birds, whereas the accelerometer recorded vocalizations only produced by the carrier. Accelerometer signals consisted of high-quality audio recordings up to almost 5 kHz, well suited for perfect classification of vocal elements even in the midst of other birds vocalizing.
We studied 3 groups of 4 male birds each and recorded their vocalizations during several days in a sound-proof chamber. We collected data during 2.5 morning hours when vocalization density was highest. Every other day during the 2.5 morning hours we introduced a female to the group

to study the development of vocal interactions between the males and the female.
We measured the strength of pairwise interactions between birds by computing cross-correlation (CC) functions of the number of selected vocal elements in sliding 250 ms time windows. To create a linear ranking of birds we also computed CC functions between calls in a given bird and calls in all other birds, to sort the birds by the times of their CC maxima (within a range of time lags typically smaller than 200 ms). This ranking reflects the average order of calls in the entire group.
We have found that communication networks determined by CCs in zebra finch groups have a stable hierarchical structure. The networks form rapidly in newly created groups and strengthen over the course of several days, where the strengthening is manifested in faster and more reliable call-call responses. Singing behaviors differed between individuals. Some animals tended to sing together whereas others avoided that tendency. Introduction of the female strongly decreased the number of songs produced by males and changed the hierarchy of their call interactions within the entire group. However, after female removal the original hierarchy recovered in most cases. These findings of rapid hierarchy formation suggest the existence of strongly pairwise attractive rules for the formation of communication networks that depend on the group composition as a whole.

Disclosures: A.L. Vyssotski: None. **V.N. Anisimov:** None. **A.V. Latanov:** None. **R.H.R. Hahnloser:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.11/QQ17

Topic: D.02. Auditory

Support: 1F32DC012449-01A1

Title: Modulation of spontaneous and sensory-evoked synaptic dynamics in A1 during auditory discrimination tasks in mice

Authors: *M. J. MCGINLEY[1], S. V. DAVID[2], D. A. MCCORMICK[1];
[1]Neurobio., Yale Univ., New Haven, CT; [2]Oregon Hearing Res. Ctr., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Sensory cortex is capable of remarkable plasticity in the coding of stimuli, particularly during complex behaviors. The dynamics of sensory coding and its plasticity have been extensively studied with extracellular single unit recordings and lower resolution measures of

population activity (e.g. EEG, LFP, and fMRI). However, whole-cell recordings of the membrane potential dynamics of cortical neurons have largely been restricted to anesthetized or passively awake and unengaged animals, yielding diverse and apparently conflicting results. For example, membrane potentials in some cortical areas of awake cats and mice are depolarized in a persistent UP state (Steriade, Timofeev, and Gernier, J Neurophysiol 85:1969-85, 2001; Haider, Hausser, and Carandini, Nature 493:97-100, 2013). However, other areas of the mouse cortex show different patterns of activity. Membrane potentials in auditory cortex of awake mice are hyperpolarized in a persistent DOWN state (Hromadka, Zador, and DeWeese, J Neurophysiol 109: 1989-95, 2013) and in barrel cortex of quietly awake mice they exhibit slow-oscillations reminiscent of sleep (Crochet and Petersen, Nat Neurosci 9:608-10, 2006). Task related plasticity in the membrane potential dynamics of cortical neurons has not been demonstrated.
Here, we perform whole-cell recordings in the primary auditory cortex of mice engaged in two auditory discrimination tasks of varying difficulty while head-fixed on a cylindrical treadmill. In the simpler task, mice are trained to distinguish complex noise stimuli (temporally orthogonal ripple combinations; TORCs) from a pure tone. Sounds are presented in trial blocks with 1-6 TORCs followed by a tone. The pitch of the tone is fixed during each training session but is varied randomly day-to-day. In the more difficult task the tone is embedded in a TORC with variable signal-to-noise ratio between trials. These tasks result in rapid changes in gain and shape of receptive fields for extracellular recordings in ferrets (Atiani, Elhilali, David, et al., Neuron 61:467-80, 2009). Mice were able to learn both tasks and perform them reliably for up to 1000 trials with sigmoidal psychometric curves and during whole-cell recordings. We find that membrane potentials are stable and frequently depolarized when animals are engaged in the difficult task, whereas they undergo slow fluctuations or sustained hyperpolarization when unengaged or unchallenged. Investigation of the effects of task performance on receptive fields is ongoing.

Disclosures: M.J. McGinley: None. **S.V. David:** None. **D.A. McCormick:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.12/QQ18

Topic: D.02. Auditory

Support: NIDCD R21 DC012894

Title: Layer 6 corticothalamic projections actively maintain sound selectivity in the lemniscal subdivision of the medial geniculate body but provide gain control to a non-lemniscal subdivision

Authors: *A. R. CLAUSE[1,2], D. B. POLLEY[1,2];
[1]Eaton-Peabody Lab., Massachusetts Eye & Ear Infirmary, Boston, MA; [2]Otology and Laryngology, Harvard Med. Sch., Boston, MA

Abstract: Corticothalamic (CT) projections originating from layers 5 and 6 of the cortex (L5 and L6, respectively) make up the largest component of the auditory corticofugal system and provide a massive, specifically patterned input to the lemniscal and non-lemniscal subdivisions of the medial geniculate body (MGB). L5 and L6 CT projections have distinct innervation patterns, axon morphology, and functional properties. However, conventional approaches for neuronal silencing affect both L5 and L6 rather than isolating each laminar component. Accordingly, the effects of AC silencing on sound representations in the MGB have been mixed and difficult to interpret.

We have taken a chemical-genetic approach to selectively and reversibly inactivate L6 neurons in the mouse auditory cortex (AC) while simultaneously recording sound-evoked activity in the medial, dorsal, and ventral subdivisions of the MGB. The AC of both hemispheres was infected with a cre-dependent adeno-associated viral vector that drove expression of a mutant acetycholine receptor only in L6 pyramidal neurons. When activated by the systemic injection of its otherwise inert synthetic ligand, this receptor elicits robust membrane hyperpolarization. Unlike optogenetics-based inactivation, this approach, known as DREADDs, provides long-lasting neuronal inhibition (4 hrs in vivo) throughout a large, irregularly shaped expression area without the risk of tissue toxicity.

By chronically implanting multi-channel silicon probes mounted on a lightweight microdrive, we were able to record from neurons throughout the different subdivisions of the MGB in freely moving mice before, during, and after L6 inactivation. We presented a large battery of sound stimuli to examine the influence of L6 feedback on a various aspects of sound-encoding, such as pure tone tuning, conspecific vocalization responses, and representations of signals in noise. Preliminary results demonstrate that the L6 inactivation was associated with increased sound-evoked firing rates in the medial, but not ventral, subdivision, while tuning quality decreased in the ventral, but not medial, subdivision. CT feedback has been implicated in a wide array of functions, ranging from the dynamic modification of sensory filters to the adaptive re-mapping of sound localization cues, and is likely to be differentially active based on ongoing cognitive demand. Accordingly, current efforts are focused on comparing the effects of L6 inactivation when mice are in three listening conditions: anesthetized, awake and passively listening, and awake and actively monitoring sound cues to avoid an aversive stimulus.

Disclosures: A.R. Clause: None. **D.B. Polley:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.13/QQ19

Topic: D.02. Auditory

Support: CIHR CSA Award

CONACYT fellowship

IPN Graduate award

Title: Enhanced but dysregulated experience-dependent plasticity in the aged brain due to reduced inhibition

Authors: *M. CISNEROS-FRANCO[1], E. DE VILLERS-SIDANI[2];
[1]McGill Univ. Integrated Program In Neurosci., Montreal, QC, Canada; [2]Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: During an early developmental period also known as the "critical period" (CP), cortical inhibitory networks are immature and passive exposures to environmental sounds readily shape the frequency tuning map of the primary auditory cortex (A1) (de Villers-Sidani et al., 2007). Maturation of cortical inhibition ultimately closes the CP, and early experience-dependent alterations in A1 tuning become consolidated and resistant to passive sound exposures. Moreover, a minimum inhibitory tone is necessary for the maintenance of this representational stability in the adult cortex (Pizzorusso et al., 2002).
In aged rats and humans, although learning is still possible, the process is usually slower and effects short-lived (Boyke et al., 2008; de Villers-Sidani et al., 2010). Notably, the inhibitory elements that are associated with the regulation of plasticity during early development are reduced in the aged brain (Caspary et al., 2008). This altered milieu could result in a progressive increase in representational instability in the aged A1, potentially contributing to the reduced effectiveness of learning in older individuals. Here we tested this hypothesis by comparing the effect of a short passive tone exposure on A1 frequency representation in young adult and aged rats. We found that such exposure resulted in a significant distortion of A1 frequency tuning in the older but not in the younger group. Additionally we found that such distortions could readily be erased by a subsequent exposure to a different tone. Finally we administered the GABA-A potentiator diazepam in the aged group during the exposure and found that it made A1 once again resistant to passive alterations in frequency tuning.
Our findings show that experience-dependent plasticity is paradoxically enhanced but dysregulated in the aging brain likely due due to an age-related reduction in inhibition. Such

instability could have a direct negative impact on the acquisition and persistence of learning in the aged brain.

Disclosures: M. Cisneros-Franco: None. **E. de Villers-Sidani:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.14/QQ20

Topic: D.02. Auditory

Support: NIDCD Grant DC-010013 to N.M.W.

Title: Learning strategy shift accounts for renormalization of sensory map plasticity

Authors: G. A. ELIAS[1,2], K. M. BIESZCZAD[1,2], *N. M. WEINBERGER[1,2];
[1]Cntr for the Neurobio of Learning & Memory, Univ. of California, Irvine, IRVINE, CA;
[2]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Representational plasticity (RP) is a *systematic learning induced bias in the representation of sensory stimuli along a dimension*. In the primary auditory cortex (A1) the degree of RP involves the learning strategy employed (Berlau & Weinberger, 2008, *Neurobiol Learn Mem*). A learning strategy to bar-press for reward from tone onset until receiving an error signal after tone offset (TOTE; tone-onset to error) produces an expanded representational area for the tone (Bieszczad & Weinberger, 2010, *Neurobiol Learn Mem*). However, expansions dissipate ("renormalize") with over-training (weeks) (Reed et al., 2011, *Neuron*). As learning strategy plays a critical role in the induction of RP, it may help explain renormalization. Therefore, we trained two groups of rats for different amounts of time in a discrimination task: one tone (CS+) signaled water reward contingent on a bar-press and two tones did not (CS−). Group 1 (n = 11) was trained to asymptotic correct performance (7−15 days, mean = 10) while Group 2 (n = 10) was over-trained for an additional two weeks. To promote use of a TOTE strategy, bar-presses made during and immediately following CS+ offset were rewarded. Rats in both groups learned to use the TOTE strategy and were indistinguishable during the first two weeks, in amounts of TOTE strategy use and levels of asymptotic performance. Over-training in Group 2 led to a decline in TOTE use, which was replaced by a strategy where bar-pressing, still initiated at tone-onset, was stopped before generating an error signal, *i.e.*, animals internalized the reward contingencies (iTOTE). Terminal mapping after training revealed that Group 1 exhibited CS+ specific area expansions, replicating prior findings. In contrast, Group 2 trained to high levels of iTOTE use had no expansions. Importantly, the amount of TOTE use was

positively correlated with the amount of CS+ area ($r = 0.50$, $p < 0.05$), while iTOTE use was negatively correlated with CS+ area ($r = -0.58$, $p < 0.01$). Thus renormalization appears due to a change in learning strategy, rather than a passive dissipation of area gain. As greater use of the over-training strategy led to greater loss of CS+ area, renormalization may reflect a shift from cortically-dependent cognitive memory to a more habit-like, possibly subcortically dependent, form of behavioral response (*e.g.*, Mishkin & Petrie, 1984, *Neuropsych of Memory*).

Disclosures: G.A. Elias: None. **K.M. Bieszczad:** None. **N.M. Weinberger:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.15/QQ21

Topic: D.02. Auditory

Support: ONR Grant

NIH Grant

Title: Differential neuronal responses in ferret frontal cortex during performance of positive and negative reward versions of an auditory long-term memory task

Authors: *J. B. FRITZ, S. A. SHAMMA, P. YIN;
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Abstract: An earlier study in ferret frontal cortex (FC) revealed persistent changes in neuronal responses to novel auditory target stimuli over a time course of minutes to hours following performance of auditory discrimination tasks (Fritz et al 2010). In order to explore the role of the FC in auditory memory, we developed a new auditory task that depends on information retrieval from an auditory long-term memory (LTM) store. Ferrets were trained to classify single tone-bursts into three frequency ranges (Low, Middle and High). Three animals were trained on a positive reinforcement (PR) task version, in which they learned to approach a waterspout for reward (Go-response) when the tone-burst fell in the Middle frequency range, and to avoid a time-out by not licking the waterspout after tone-bursts in either the Low or High frequency range (No-Go response). Five additional ferrets were trained on a conditioned avoidance (CA) version of the task, in which they received a mild shock if they licked to tones with frequency in the Middle range, but could lick freely through the tones in both Low and High range. Each trial thus consisted of the presentation of a single tone-burst that occurred randomly within one of the frequency zones, and the animal's response to this tone. After learning the frequency range

classification LTM task, 4 ferrets (2 in PR and 2 in CA) also learned a similar 3-zone classification for different rates of amplitude modulation (AMR) of white noise. Each daily session consisted of 100-200 trials on one or both tasks. After animals were trained, they were implanted with head-posts to secure head position during task performance. We recorded chronically from the FC of head-fixed ferrets, using a multiple electrode system (Alpha Omega). Preliminary neurophysiological results from the PR pitch LTM task indicate the presence of two major neuronal response types in FC that were strongly activated during performance and selectively preferred tonal frequencies that corresponded to behavioral choices rather than acoustic zones. FC neurons responded to either (a) the Middle range "Go" stimuli or (b) both Low and High range "No-go" tone-burst stimuli (Yin et al 2012). In contrast, during the CA pitch LTM task we only observed FC neuronal responses to the Middle range "No-go" tone bursts, even though the animal was performing the same pitch discrimination task. A similar response pattern was found during the AMR task in the same neuron population. These preliminary results provide insights into how the FC encodes, represents, classifies and retrieves the associative meaning of sensory stimuli during performance of auditory LTM pitch or AMR tasks under different reward conditions.

Disclosures: J.B. Fritz: None. **S.A. Shamma:** None. **P. Yin:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.16/QQ22

Topic: D.02. Auditory

Support: DIRP, NIMH/NIH

Title: Activity of auditory cortical neurons in monkeys performing a short-term memory task

Authors: *B. H. SCOTT[1], P. YIN[2], M. MISHKIN[1];
[1]Lab. Neuropsychol, NIMH, BETHESDA, MD; [2]Inst. for Systems Res., Univ. of Maryland, College Park, MD

Abstract: Short-term memory (STM) for visual stimuli has been shown to engage both prefrontal cortex (PFC) and the modality-specific cortical areas that support visual perception. Although monkeys can perform auditory STM tasks, their ability is limited relative to that in vision, and appears to depend on retention of a stimulus trace in a passive form of STM. The neural underpinnings of this putative trace are unknown, but by analogy to sensory memory in vision and touch, they are likely to engage non-primary auditory cortex, e.g., the rostral superior

temporal plane and gyrus, components of the ventral auditory processing stream that provide input to the PFC. We recorded single-unit activity across these regions while monkeys performed a serial delayed-match-to-sample (DMS) task. On each trial, the monkey grasped a bar to initiate the presentation of a sample sound (~300 ms duration), followed by 0-2 nonmatch sounds (delay interval ~1 s), before the sample was presented again as a match; the monkey released the bar to indicate a match. Among 280 sound-responsive neurons in 3 animals, we identified two phenomena potentially associated with mnemonic tasks. First, 35% of units exhibited a sustained change in firing rate (relative to the pre-trial baseline) during the delay interval (17% excitation, 18% suppression). Second, the auditory response was modulated by task context in 20% of units, with 7.5% showing match enhancement (relative to the sample presentation), and 12.5% showing match suppression. The prevalence of excitatory and suppressive effects differed between early and late phases of the trial: delay and match suppression were observed throughout the trial, but the proportion of units exhibiting delay or match excitation declined significantly after the presentation of the first nonmatch sound. The decline in excitatory response modulation following the first nonmatch sound coincides with a marked increase in behavioral error rate, suggesting that these signals may aid match detection. However, in the spiking activity there was no evidence of a stimulus-specific trace spanning the delay interval - analysis of firing rate in a sliding time window revealed that the variance explained by stimulus identity decayed to zero ~300 ms after stimulus offset for the population on average (whether during the task or during passive listening). The dynamics of spiking activity in the auditory ventral stream are consistent with characteristics of DMS performance, but additional sub-threshold mechanisms are likely to support the neural trace on which STM depends.

Disclosures: B.H. Scott: None. **P. Yin:** None. **M. Mishkin:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.17/QQ23

Topic: D.02. Auditory

Support: Wellcome Trust Project Grant (WT092606AIA)

Title: Cortical oscillations and spiking activity associated with Artificial Grammar Learning in the monkey auditory cortex

Authors: *Y. KIKUCHI, A. ATTAHERI, A. MILNE, B. WILSON, C. I. PETKOV;
Inst. of Neurosci., Newcastle Univ. Med. Sch., Newcastle Upon Tyne, United Kingdom

Abstract: Artificial Grammars (AG) can be designed to emulate certain aspects of language, such as the structural relationship between words in a sentence. Towards developing a primate model system to study potential language precursors at the neuronal level, we obtained evidence that monkeys can learn relationships in sequences of nonsense words generated from an auditory AG and used functional MRI (fMRI) to study the brain regions engaged (Wilson et al., SFN, 2011). Here, we ask how monkey auditory neurons evaluate the within-word acoustics and/or between-word sequencing relationships, and whether these aspects engage theta and gamma oscillations, which are critical for speech processing in human auditory cortex (e.g., Giraud & Poeppel, Nat. Nsci. 2012). We recorded local-field potentials (LFPs) and single-unit activity (SUA) from 4 fMRI localised auditory core (A1 & R) and lateral belt (ML & AL) subfields in two Rhesus macaques (124 sites). During each recording session, the monkeys ware first habituated to exemplary sequences generated by the AG. We then recorded neuronal activity in response to identical nonsense words, either in the context of a sequence that followed the AG structure ('correct') or one that violated its structure ('violation'). In response to nonsense words, the LFP power significantly increased in a broad range of frequency bands (4-100 Hz), including at theta (4-10Hz) and low (30-50Hz) and high (50-100Hz) gamma frequencies. We also observed a consistent increase in the inter-trial phase coherence, in particular in the theta band. Moreover, a substantial proportion of the LFP sites showed differential responses to the nonsense words depending on whether the word was in the context of a 'correct' or 'violation' sequence. This was observed in a considerable proportion of sites in the theta (35/124 sites), low gamma (37/124) and high-gamma (25/124) bands. Moreover, the proportion of such sequence-context sensitive sites increased from core to lateral belt auditory fields (LFP: 18% vs. 82%; SUA: 39% vs. 61%). We provide evidence that monkey auditory neuronal responses, including theta and gamma oscillations, are associated with both the processing of nonsense words and the relationship between the words, as governed by the AG. These nonhuman primate results likely reflect domain general evolutionarily conserved neuronal processes, rather than those that are language specific in humans.

Disclosures: Y. Kikuchi: None. **A. Attaheri:** None. **A. Milne:** None. **B. Wilson:** None. **C.I. Petkov:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.18/QQ24

Topic: D.02. Auditory

Support: FNS 100014_125050 James et al.

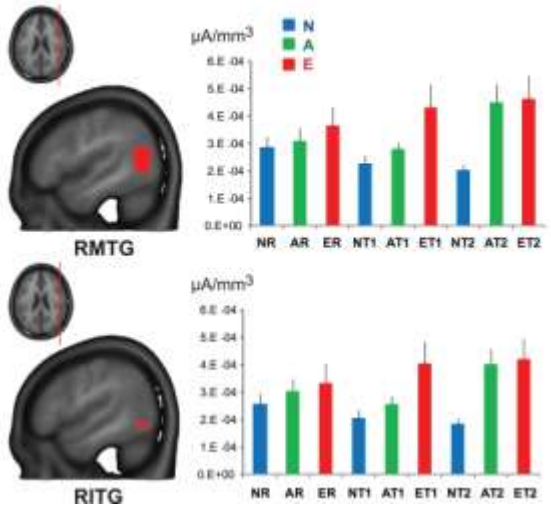
Title: ERP microstates and source imaging reveal progressive changes in cerebral processing of musical syntax with level of musical expertise

Authors: *C. E. JAMES[1,2], M. S. OECHSLIN[5], D. VAN DE VILLE[3], F. LAZEYRAS[3], D. BAVELIER[2], C. MICHEL[4];
[1]Univ. of Applied Sci. Western Switzerland, Geneva, Switzerland; [2]Fac. of Psychology and Educational Sci., [3]Fac. of Medicine, Dept. of Radiology and Med. Informatics, [4]Fac. of Medicine, Dept. of Fundamental Neurosciences, Univ. of Geneva, Geneva, Switzerland; [5]Intl. Normal Aging and Plasticity Imaging Ctr., Univ. of Zurich, Zurich, Switzerland

Abstract: While recording high density EEG, non-musician (N), amateur (A), and expert (E) musicians assessed a series of specifically composed string quartets with hierarchically manipulated endings. At musical closure, we implemented 2 levels of transgression, subtle (T1) and apparent (T2)), that differed in salience but remained both within the tonality of the piece (in-key). Pieces with transgressed endings were presented intertwined with pieces ending regularly (R). Participant groups were matched for age, sex and fluid intelligence; moreover, A and E only differed in training intensity, not in age of training onset. Behavioral sensitivity scores (d-prime) of both transgressions clearly separated participants as a function of expertise level. Classical ERP analyses exhibited significant Expertise x Transgression interaction between 200 and 600ms after stimulus onset (i.e. onset of musical closure). Amplitudes increased with expertise level, but differentially so for all three conditions (R, T1, T2). Results of spatio-temporal ERP analyses, in a 300-500ms time window after stimulus onset, nicely matched the behavioral results and yielded specific ERP topographies or "microstates" of information processing according to expertise. Expertise x Transgression x Microstate interaction showed gradual differences between levels of expertise for the incidence of certain microstates, grouping E against A & N for T1, and N against A & E for T2. Finally, computing inverse solutions (LORETA) of ERPs in this 300-500ms time window, we could show significant Expertise x Transgression interaction in the right middle and inferior temporal gyrus. Stronger activations manifested in these brain areas with increasing expertise, but for T2, the apparent transgression, A and E did not differ (Figure 1). These results strongly suggest that training intensity

progressively changes higher order cognitive brain functioning.

Figure 1: significant Expertise x Transgression interaction in the Right Middle and Inferior Temporal Gyrus (RMTG, RITG). Left panel: significant voxels superimposed on slices of MNI 152 template brain. Right panel: mean current density (μA/mm3) for each group and condition over 295-480 ms from stimulus onset. **R:** regular endings; **T1:** subtly transgressed endings; **T2:** apparently transgressed endings. **N:** non-musicans; **A:** amateur musicians; **E:** expert musicians.



Disclosures: C.E. James: None. **M.S. Oechslin:** None. **D. Van De Ville:** None. **F. Lazeyras:** None. **D. Bavelier:** None. **C. Michel:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.19/QQ25

Topic: D.02. Auditory

Support: Berufsgenossenschaft Nahrungsmittel und Gastgewerbe (Institution for statutory accident insurance and prevention in the foodstuffs industry and the catering trade)

Title: Occupationally induced hearing impairment: Is the error rate during auditory pattern recognition affected and does this produce mental stress?

Authors: E. EMMERICH, *F. RICHTER;
Inst. of Physiol. I, Univ. Hosp. Jena, Jena, Germany

Abstract: The impact of noise on the inner ear causing hearing damage or hearing loss as well as risk factors of noise has been often investigated in the past. Little is known, however, about non-aural influences of noise causing mental stress in hearing-impaired people. Noise can increase heart frequency, cardiac output, and also the systemic blood pressure together with endocrine

changes.
We investigated 20 workers (aged 41-62 years) from beverage industry with occupationally induced hearing impairment. In these volunteers we recorded the EEG from 31 channels (10-20 system) particularly from the auditory cortex, together with the electrocardiogram, breathing frequency and systemic blood pressure. The EEG was analyzed for auditory evoked potentials and for changes in frequency before and after stimulus presentation. Stimuli were either short samples of recorded sounds from the machines in the beverage industry (parent stimulus), or the same sounds with very small mistakes, e.g. interruptions equivalent to broken bottles (deviant stimulus). For comparison we presented synthesizer chords in-tune or slightly mistuned, respectively. Stimuli were presented in the oddball paradigm (ratio: 4 parent, 1 deviant). The workers were asked to mark every mistake by pressing a bottom.
The workers were able to recognize nearly all mistakes when workplace sounds were presented even at reduced sound pressure, but failed when synthesizer chords were used. Interestingly, in both stimulus presentations auditory evoked potentials clearly differed between parent and deviant stimuli. Only after inaccurate workplace sounds but not after mistuned synthesizer chords the EEG frequency shifted significantly towards higher frequencies in the beta wave band. Together with higher EEG frequencies, both heart rate and systemic blood pressure increased transiently, especially when several inaccurate workplace sounds were presented in succession. The workers told on uneasiness after such a series of inaccurate sounds.
The results showed that learning of specific occupational sounds during professional life supports auditory pattern recognition and is able to compensate to some extent the hearing impairment. Regardless of the hearing impairment, inaccurate auditory patterns at the workplace cause cardiovascular and EEG frequency changes indicating mental stress. This should be considered when extra aural influences of noise are to be evaluated.

Disclosures: E. Emmerich: None. **F. Richter:** None.

Poster

549. **Auditory System: Adaptation, Learning, and Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 549.20/QQ26

Topic: D.02. Auditory

Support: NIH Grant DC006243

Title: Pitch matching vocal training paradigm with audio cuing and F0 perturbation, a software only implementation

Authors: *B. ROGERS[1], A. L. PARKINSON[1], C. R. LARSON[2], D. A. ROBIN[1,3];
[1]Res. Imaging Inst., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [3]Dept. of Communication Sci. and Disorders, [2]Northwestern Univ., Evanston, IL

Abstract: The ability to control voice fundamental frequency (F0) is essential for successful and efficient speech production. Accurate voice F0 and amplitude control depend on error correction mechanisms that process sensory feedback. Pitch perturbation techniques have often been used investigate the role of auditory feedback in F0 control.
In this paradigm participants vocalize to match the pitch of audio cues while receiving intermittent pitch shifted feedback. Participants wear over the ear headphones for the presentation of audio cues and for giving real time audio feedback. An attached microphone is used to record vocalization.
The audio cues are phrases made up of single syllables such as "TA" or "STRA". Before the session participants will vocalize each cue syllable once. The cue vocalizations are stored and use to construct the prompt phrase for each trial in the session. One of the syllables of the cue may be shifted to a higher or lower frequency.
For each trial, the participant sees a visual cue and hears an audio cue. If the second syllable of the phrase "TA TA TA TA" is to be vocalized at a higher pitch, the second "TA" of the visual cue is positioned higher. In the associated audio cue, the second "TA" will be pitch shifted higher. Then, they are tasked with matching the audio cue exactly. During vocalization, the audio feedback may be perturbed up or down during the one syllable that is shifted. The participant's performance on matching the cue phrase is visually presented immediately as a set of matching bars to indicate how close each vocalized syllable was to the cue.
This paradigm is implemented in C++ using free or open source components. It runs on both Windows and Macintosh computers. A friendly graphical user interface is created using the QT framework (Nokia). The RtAudio API (McGill University) is used as a platform independent audio interface. The real time audio pitch shifting uses the smbPitchshift C++ FFT routine (Stephan M. Bernsee). The pitch of the captured vocalizations is determined by spawning an instance of Praat (University of Amsterdam). Latencies of the pitch shifted audio of less than 25ms have been measured using the built in audio of both Macintosh and Windows computers. Latencies of around 15ms have been measured using a fast PCI audio card.
This paradigm can be useful in studying error detection/correction, efference copy, and feedforward/feedback mechanisms. Target populations include the elderly, those with Parkinson's disease, stroke, or vocal disorders, as well as professional singers and musicians. The paradigm fits well with behavioral, imaging and electrophysiology studies.

Disclosures: B. Rogers: None. **A.L. Parkinson:** None. **C.R. Larson:** None. **D.A. Robin:** None.**Poster**

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.01/RR1

Topic: D.03. Multisensory

Title: No unique neural network for abstract coding of audiovisual speech

Authors: *N. MALFAIT[1], P. FONLUPT[2], L. CENTELLES[3], B. NAZARIAN[1], L. BROWN[4], A. CACLIN[2];
[1]CNRS / INT, Marseille, France; [2]INSERM / U1028, Lyon, France; [3]Aix Marseille Univ., Marseille, France; [4]Trent Univ., Peterborough, ON, Canada

Abstract: How are we able to easily and accurately recognize speech sounds despite the lack of acoustic invariance. One proposed solution to this long-standing question has been the existence of a neural representation of speech syllable perception that transcends its sensory properties. In the present fMRI study, we used two different audiovisual speech contexts both intended to identify brain areas whose levels of activation would be conditioned by the speech percept independent from its sensory source information. We exploited McGurk audiovisual fusion to obtain short oddball sequences of syllables that were either a) acoustically different but perceived as similar, or b) acoustically identical but perceived as different. We reasoned that if there is a single network of brain areas representing abstract speech perception, this network would show a reduction of activity when presented with syllables that are perceived as similar and an increase in activity when presented with syllables that are acoustically similar but perceived as distinct. Consistent with the long-standing idea that speech production areas may be involved in speech perception, we found that frontal areas were part of the neural network that showed reduced activity for sequences of perceptually similar syllables. Another network was revealed, however, when focusing on areas that exhibited increased activity for perceptually different but acoustically identical syllables. This alternative network included temporal auditory areas but no left frontal activations. In addition, our findings point to the importance of subcortical structures much less often considered when addressing issues pertaining to perceptual representations.

Disclosures: N. Malfait: None. **P. Fonlupt:** None. **L. centelles:** None. **B. Nazarian:** None. **L. Brown:** None. **A. Caclin:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.02/RR2

Topic: D.03. Multisensory

Support: Royal Academy of Music, Aarhus/Aalborg, Denmark

Ministry of Culture, Denmark

The Danish National Research Foundation's Grant to the Center of Functionally Integrative Neuroscience

Title: Tract-based spatial statistics of absolute pitch: white matter differences in association fibers

Authors: *E. A. GARZA-VILLARREAL[1,2,3], A. DOHN[3], M. CHAKRAVARTY[4,5,7], M. HANSEN[3], J. P. LERCH[6], P. VUUST[3,8];
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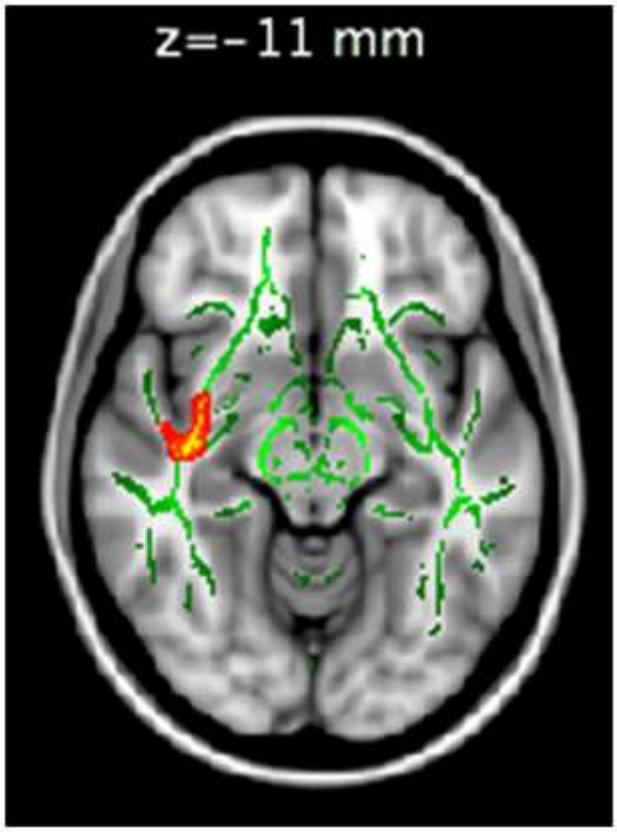
Abstract: The ability to identify a musical pitch without a reference is called 'absolute pitch' (AP). In this study we investigated white matter differences and its relation to the AP ability by performing tract-based spatial statistics (TBSS) and deterministic tractography to analyze diffusion tensor images. Thirty-five musicians entered the study, divided into two groups: AP possessors and musicians with no absolute pitch. The images were acquired using a 3 T GE MR system with an eight-channel Invivo head coil, with a high-resolution T1 SPGR 3D volume and a diffusion tensor imaging (DTI) sequences. The DTI images were preprocessed using FMRIB's FSL. The statistical analysis of the FA data was carried out using TBSS. After the overall analysis, we transformed the TBSS results back to native space, extracting the individual ROIs derived from the significant cluster (tbss_cluster) and we calculated mean area FA (maFA) in each. We then performed correlation analysis between each participant's maFA and a pitch identification test to confirm the relationship between AP ability and the white matter group cluster. Finally, we performed tractography in each subject using Diffusion Toolkit (DTK) and TrackVis software and the individual tbss_cluster as the seed. The TBSS analysis showed that APs had higher FA values compared to the non-APs ($p<.01$; TFCE- corrected) in a single significant cluster located in the right temporal lobe's sub-gyral white matter (center of gravity: x=38.4, y= -11.6, z= -12.5), within the path of the inferior fronto-occipital fasciculus, the uncinate fasciculus and the inferior longitudinal fasciculus. There was a significant positive correlation between the maFA and PIT ($r = .57$, $p < .001$). We determined that the main fiber bundles corresponded to association fibers, namely the inferior fronto-occipital fasciculus, the uncinate fasciculus and the inferior longitudinal fasciculus. In this study we show that the APs

have greater FA values than non-APs in the white matter within the right temporal lobe, and its FA value was strongly related to the AP ability.



Disclosures: E.A. Garza-Villarreal: None. **A. Dohn:** None. **M. Chakravarty:** None. **M. Hansen:** None. **J.P. Lerch:** None. **P. Vuust:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.03/RR3

Topic: D.03. Multisensory

Support: NIH Grant R01EY021184

NSF Grant BCS-1029084

NIMH Grant 7P50MH086385

NINDS Grant 2T32NS047987

Title: Anatomical and functional networks underlying audio-visual integration

Authors: *D. BRANG[1], J. ZWEIG[1], Z. J. TAICH[2], J. MISHRA[3], S. SUZUKI[1], S. A. HILLYARD[2], V. S. RAMACHANDRAN[2], M. GRABOWECKY[1];
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Abstract: Our senses interact in daily life through multisensory integration, facilitating perceptual processes and behavioral responses. Anatomical and functional connectivity have been suggested as mechanisms underlying the ability to integrate information across the senses. Here we examined the role of both forms of connectivity in the modulation of visual processes by auditory cues. Multisensory interaction was quantified using the "sound-induced illusory flash" paradigm, in which a single visual flash paired with two auditory beeps frequently generates the novel percept of an illusory second flash. The role of anatomical connectivity was assessed by examining the relationship between the perception of an illusory second flash and individual differences in white matter connectivity (as assessed by diffusion tensor imaging) on 27 participants. Results revealed a significant positive correlation between the experience of an illusory second flash and enhanced anatomical connectivity (fractional anisotropy) between auditory and visual cortices, suggesting that increased connectivity increases auditory-visual interactions. A similar individual differences approach was applied to EEG phase and coherence data in a separate group of 28 subjects to examine the role of functional connectivity. Interestingly, the experience of an illusory second flash was significantly associated with decreased coherence between auditory and visual cortices, possibly due to increased inhibition of auditory cortex by visual cortex reducing these auditory-visual interactions. Thus, stronger anatomical connectivity increased, but stronger functional connectivity decreased auditory-visual interactions during this illusion. Altered multisensory processes have been identified in numerous clinical conditions in addition to healthy aging highlighting the need to understand the relationship between individual differences in multisensory processing and those of functional and anatomical connections.

Disclosures: D. Brang: None. **J. Zweig:** None. **Z.J. Taich:** None. **J. Mishra:** None. **S. Suzuki:** None. **S.A. Hillyard:** None. **V.S. Ramachandran:** None. **M. Grabowecky:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.04/RR4

Topic: D.03. Multisensory

Support: NSF GRFP DGE-0903443

NIH/NCATS KL2TR000102

NSF-OISE-0730255

R01 EY018923-03A1

Title: Meta-analytic connectivity modeling (MACM) of anterior vs. posterior superior temporal sulcus

Authors: *L. C. ERICKSON, J. P. RAUSCHECKER, P. E. TURKELTAUB;
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Abstract: *Introduction*: The superior temporal sulcus (STS) is implicated in many functions, including audiovisual processes (Hein and Knight, 2008). Yet STS functional organization related to auditory and visual streams, including dorsal vs. ventral distinctions is not well understood. Auditory models (Rauschecker and Scott, 2009) predict that posterior STS mainly interacts with dorsal-stream regions, whereas anterior STS mainly interacts with ventral-stream regions. To examine this possibility, we conducted MACM (Zald et al., 2012) analyses that assessed which brain areas consistently coactivated with anterior, middle or posterior STS across studies in BrainMap (brainmap.org).
Methods: Regions of interest (ROIs) were created using the lpba40 atlas: left anterior STS (LaSTS), left middle STS (LmSTS), left posterior STS (LpSTS), right anterior STS, right middle STS and right posterior STS. Studies of normal subjects, reporting foci in STS ROIs were obtained from BrainMap (Sleuth). Activation likelihood estimation (ALE) analyses were conducted (GingerALE 2.1). To match sensitivity across ROIs, we equated active voxels across each ALE analysis (min. threshold of FDR $q < 0.05$, cluster threshold >100 mm^3). ALE subtractions isolated connectivity for specific STS regions (e.g., LpSTS – (LaSTS + LmSTS)).
Results: 1185 experiments and 14532 subjects were found. In general, areas associated with sensorimotor processing (e.g., parietal and premotor areas) coactivated with LpSTS more often than with other left STS regions. Areas associated more closely with ventral-stream function (e.g., hippocampus) coactivated more often with LaSTS than other left STS regions. There was some dorsal/ventral stream connectivity overlap and other coactivations were present. Some unpredicted patterns were observed (e.g., topographical coactivations with left STS and fusiform gyrus). Coactivation patterns were found for right STS.
Conclusions: In general, these findings suggest that left pSTS coactivates mainly with dorsal-stream regions, whereas aSTS coactivates more with ventral-stream regions. Examining connectivity patterns further may provide insight into STS organization and processing.
Hein G, Knight RT (2008) Superior temporal sulcus-It's my area: or is it? J Cogn Neurosci 20:2125-2136.

Rauschecker JP, Scott SK (2009) Maps and streams in the auditory cortex: nonhuman primates illuminate human speech processing. Nat Neurosci 12:718-724.
Zald DH, McHugo M, Ray KL, Glahn DC, Eickhoff SB, Laird AR (2012) Meta-analytic connectivity modeling reveals differential functional connectivity of the medial and lateral orbitofrontal cortex. Cereb Cortex epub Oct 4 2012.

Disclosures: L.C. Erickson: None. **J.P. Rauschecker:** None. **P.E. Turkeltaub:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.05/RR5

Topic: D.03. Multisensory

Support: NIH NEI 1R01 EY019684-01A1

NSF IIS-1208203

Title: Functional cross-subject mapping predicts brain activity to novel natural movies and speech

Authors: *N. Y. BILENKO[1], A. G. HUTH[2], S. NISHIMOTO[2], J. L. GALLANT[3];
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Abstract: Individual fMRI responses are highly variable, both anatomically and functionally. This variability makes it difficult to compare individual data in voxel-based fMRI analyses. Individual variability may be especially problematic when comparing data across modalities, such as when comparing cortical representations of natural speech and movies. Here we introduce a technique of functional cross-subject mapping that uses similarity of fMRI responses across individuals to reveal the underlying dimensions of cortical representation of natural stimuli within and across experiments. We show that this method can successfully predict individual subject responses to novel natural movies and speech, both within and across modality.
We recorded fMRI responses from four human subjects while they watched two hours of silent natural movies during steady fixation, and again later while they listened to two hours of natural speech. We used regularized kernel canonical correlation analysis with cross-validation to compute the functional mappings across individual subjects. First, two functional cross-subject mappings were estimated, one for natural movies, and another for natural speech. Ninety percent of the subjects' responses in each experiment were used to compute each of the functional

mappings. Then, the held-out ten percent of each subject's responses in each experiment were predicted by projecting the other subjects' held-out responses through each of the computed mappings. The accuracy of each mapping was quantified within and across experiment by computing the correlations of each subject's predicted responses with their actual fMRI responses.
Within-experiment predictions for the natural movie experiment were most accurate in the early and higher visual cortex, as well as brain areas associated with spatial attention and eye movements. Within-experiment predictions for the natural speech experiment were most accurate in the auditory cortex, temporal-parietal junction, superior temporal sulcus, and in frontal brain regions. Across-experiment predictions were highest in semantically selective visual areas, precuneus, parts of the auditory cortex, and frontal regions.
These findings demonstrate that the functional cross-subject mapping method accurately predicts each person's brain activity to novel natural stimuli, based solely on brain activity measured in different individuals. Furthermore, we show that this method can generalize across modalities, since a functional cross-subject mapping based on natural movie stimuli can be used to predict novel brain responses to natural speech stimuli and vice versa.

Disclosures: N.Y. Bilenko: None. **A.G. Huth:** None. **S. Nishimoto:** None. **J.L. Gallant:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.06/RR6

Topic: D.03. Multisensory

Support: Bernstein Center for Computational Neuroscience - Tuebingen

University of Birmingham

Title: The left prefrontal cortex controls information integration by combining bottom-up inputs and top-down predictions

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Abstract: In the natural environment our senses are bombarded with many different signals. To form a coherent percept, the brain should integrate signals originating from a common source and segregate signals from different sources. This psychophysics-fMRI study investigated how

the human brain combines bottom-up inputs (i.e. congruent VS incongruent signals) and top-down prior predictions (i.e. common source prior) to infer whether sensory signals should be integrated or segregated.
Sixteen participants were shown audio-visual movies of congruent (e.g. visual «Ti» with auditory /Ti/), incongruent (e.g. visual «Ti» with auditory /Pi/) and McGurk syllables (e.g. visual «Ki» with auditory /Pi/, which can be fused into the illusionary percept "Ti"). Critically, we manipulated participants' top-down predictions (i.e. common source prior) by presenting the McGurk stimuli in a series of congruent or incongruent syllables. On each trial, participants reported their syllable percept in forced choice procedure with 6 response options.
At the behavioural level, participants were more likely to fuse auditory and visual signals of a McGurk trial into an illusionary percept in congruent relative to incongruent contexts. This response profile indicates that participant's prior top-down predictions (i.e. common source prior) influence whether or not they integrate sensory signals into a coherent percept.
At the neural level, incongruent relative to congruent bottom-up inputs increased activations in a widespread left-lateralised fronto-parietal network. The left prefrontal activations also increased for McGurk trials, when participants selectively reported their auditory percept and did not fuse auditory and visual McGurk signals into a unified percept. Critically, this effect was enhanced for incongruent contexts when participants expected that sensory signals are incongruent and needed to be segregated.
Collectively, our results demonstrate that the left inferior frontal sulcus determines whether sensory signals should be integrated or segregated by combining (i) top-down predictions generated from prior incongruent trials with (ii) bottom-up information about sensory conflict in the incoming signals. Furthermore, it exerts top-down control that enables participants to process sensory signals independently and selectively report their percept in one sensory (i.e. here auditory) modality.

Disclosures: G. Remi: None. **U. Noppeney:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.07/RR7

Topic: D.03. Multisensory

Support: NIH Grant NIDCD F31 DC011970 to JC

NIH Grant NIDCD RO1 06257 to AS

Title: Cross-modal re-organization in adults with mild-moderate hearing loss

Authors: *J. D. CAMPBELL, L. DURKEE, A. SHARMA;
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Abstract: Sensory deprivation has been shown to elicit cross-modal re-organization in sensory cortices. Specifically, deaf adults show activation of auditory cortex in response to visual stimulation, indicating recruitment of auditory cortex for visual processing. Such cross-modal re-organization may lead to a competition of available resources if auditory input is re-introduced via amplification or electrical stimulation. Though cortical re-organization has been studied extensively in deafness, there is an absence of research investigating the degree of hearing loss at which cross-modal re-organization is initiated. It is possible that cortical plasticity may be initiated before profound hearing loss, or deafness, occurs. Given the lack of knowledge concerning the relationship between degree of hearing loss and cortical re-organization, our goals in this study were to 1) determine if visual cross-modal plasticity occurs in adults in the early stages of hearing loss (mild-moderate severity), 2) examine changes in auditory cortical re-organization that accompany early-stage hearing loss in adults, and 3) examine if cortical changes are related to auditory behavioral outcome. High-density EEG was recorded in 8 adults with normal hearing and 9 adults with mild-moderate hearing loss while passively watching visual grating stimuli, followed by passive listening to auditory speech stimuli. Auditory speech perception performance in background noise was also measured. Finally, source localization analysis was performed on group cortical visual and auditory evoked potentials (VEP and AEPs) in order to visualize re-organization. The hearing loss group showed three main findings: 1) VEP and AEP peak component amplitudes were significantly increased, 2) VEP component latency showed significant increases while AEP component latency showed significant decreases, 3) Auditory cortical areas were active in visual processing and frontal cortical areas were active in auditory processing, and 4) Amplitude and latency changes in the VEP and AEP components were significantly correlated to degree of hearing loss as well as the ability to perceive speech in background noise. These findings demonstrate that cortical re-organization begins in early-stage hearing loss for both visual and auditory modalities, and that cortical re-organization is related to poorer auditory performance in challenging environments.

Disclosures: J.D. Campbell: None. **L. Durkee:** None. **A. Sharma:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.08/RR8

Topic: D.03. Multisensory

Support: NIH NIDCD R01 DC0625 to A.S.

Title: Somatosensory-to-auditory cross-modal plasticity in deaf children with cochlear implants

Authors: *G. J. CARDON, A. SHARMA;
Speech, Language, and Hearing Sci., Univ. of Colorado At Boulder, Boulder, CO

Abstract: *Objective:* Mounting evidence suggests that the maturation and plasticity of the cerebral cortex may play a significant role in predicting functional performance. For instance, it appears that a deficient sensory system has the tendency to re-organize through the recruitment of deprived sensory areas by intact modalities (i.e., cross-modal re-organization), and that recruited sensory modalities seem to suffer functional deficits. Deafness is a naturally occurring instance of sensory deprivation. Due to technology, such as cochlear implants (CI), sensory stimulation can be restored to deprived systems. However, not all CI recipients exhibit favorable functional performance. Somatosensory-to-auditory (SS-A) cross-modal re-organization has been demonstrated in deafness, though never in CI children. However, because the SS and A systems are responsive to virtually the same physical phenomena (i.e., mechanical pressure in the form of oscillations), and due to the highly plastic state of the developing brain, it is plausible that SS-A cross-modal re-organization could occur in deafness and be related to functional outcome in CI children. Thus, we aimed to investigate SS-A cross-modal re-organization in CI children, and whether the degree of SS-A cross-modal re-organization was related to variability in functional abilities.
Methods: Two groups of children were recruited: 1) children with normal hearing; 2) cochlear-implanted children. Each participant underwent high-density EEG testing in response to both auditory and somatosensory stimulation. Assessment of both cortical auditory and somatosensory evoked potential (CAEP; CSEP) waveform components was performed. Current density reconstruction was also carried out to ascertain the sources of cortical activity in response to each type of stimulation.
Results: Results from both evoked potential analysis and current density reconstruction suggest that CI children, but not those with normal hearing, showed SS-A cross-modal re-organization. Additionally, CI children with poor speech perception performance presented with a greater degree of SS-A cross-modal re-organization than those with more favorable functional abilities.
Conclusions: Our findings indicate that SS-A cross-modal re-organization can occur in deaf children who use CIs. The degree of this cortical re-organization also appears to be related to functional outcome. These findings contribute to our understanding of experience-dependent compensatory cortical plasticity in humans. Furthermore, understanding, and being able to harness, cortical plasticity could lead to advancements in clinical management of CI users.

Disclosures: G.J. Cardon: None. **A. Sharma:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.09/RR9

Topic: D.03. Multisensory

Support: CFI (Canada)

NSERC (Canada)

ORF (Canada)

NNSF (China)

Title: Visual-tactile integration in the human brain: A combined EEG-fMRI study

Authors: *D. WANG[1,2,3], D. Q. MIAO[1,2], B. COE[3,4], J. GALLIVAN[3,4], G. BLOHM[3,4];
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Abstract: Multi-sensory integration is at the heart of sensory processing and is crucial for perception and action. Much research on multi-sensory integration has been carried out in monkeys primarily focusing on sub-cortical structures. To gain insight into spatio-temporal mechanisms of human multi-sensory integration in the cortex, we investigated visual-tactile integration for the guidance of eye movements.
We simultaneously recorded EEG (256 sensors) and fMRI (3T) signals in a fast event-related design from 10 subjects while performing a delayed saccade task to visual (V), tactile (T) or combined visual-tactile (congruent (VT) or incongruent (V~T)) stimuli located on their right forearm. Four stimulus locations (2 left, L1-L2; 2 right, R1-R2) were approximately equally spaced (5cm apart) centered on the visual midline and a fixation LED was positioned 5cm above the center of the stimuli. We used a 4×4 factorial design (SPM8) for the fMRI analysis with factors condition (V, T, VT, and V~T) and target location (L1, L2, R1, and R2) and statistics were FWE corrected of $p<0.001$.
Compared to baseline, we found bilateral activation in FEF, mIPS, V3 and V6 and left (contralateral) S1 during the delay period of the saccade. Comparing uni-sensory and multi-sensory stimulation (V+T>VT and V+T<VT), significant differences arose in the right (ipsilateral) middle cingulate, FEF and V3. Contrasting VT > V~T conditions, led to significant effects in the left superior and posterior middle frontal cortex (SFC, pMFC). Time-frequency response analysis of EEG signals in parietal sensors revealed a decrease in gamma-band (60-

70Hz) synchronization during the whole memory period and an increase in alpha- (10Hz) and beta-band (20Hz) synchronization 500ms into the delay period for multi-sensory stimulation. Our results show a large network of occipital, parietal, and frontal areas involved in multi-sensory visual-tactile integration. We also show specific brain areas involved in multi-sensory conflict detection (SFC and pMFC). Finally, our EEG analysis points towards multiple frequency components related to sensory, motor, and memory processing reflecting visual-tactile integration. To our knowledge, this is the first imaging study providing both high spatial and high temporal resolution for human multi-sensory integration in cortex.

Disclosures: D. Wang: None. **D.Q. Miao:** None. **B. Coe:** None. **J. Gallivan:** None. **G. Blohm:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.10/RR10

Topic: D.03. Multisensory

Support: NSF Grant EEC-0310723

NEI Grant R01 EY017707

Research to Prevent Blindness (RPB)

USC Dana and David Dornsife Cognitive Neuroscience Imaging Center

NSF Graduate Research Fellowship Program

Title: Correlation of functional and structural visual stream connectivity with V1 tactile-evoked BOLD responses in patients with retinitis pigmentosa

Authors: *S. I. CUNNINGHAM[1], J. D. WEILAND[1], P. BAO[2], B. S. TJAN[3];
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Abstract: Previous fMRI studies have demonstrated that vision loss is correlated with cross-modal responses in primary visual cortex (V1), where the amount of tactile-evoked activity increases as vision gets worse in late-blind individuals. Some studies suggest that the dorsal

visual stream (specifically visual association areas of the parietal lobe) may be a region where visuotactile information is integrated in sighted individuals, while blindness may cause both dorsal and ventral visual streams to process tactile stimuli. The purpose of this study is to determine if tactile-evoked V1 responses are associated with changes in brain functional and structural connectivity following vision loss. Four late-blind retinitis pigmentosa patients and 2 sighted subjects underwent resting-state fMRI and diffusion tensor imaging scans in a 3T Siemens MRI scanner; all subjects had previously completed an fMRI tactile-discrimination study. Regions of interest (ROIs) were selected to include voxels along both ventral and dorsal visual pathways. In order to determine the functional connectivity between pairs of ROIs in each subject, we calculated the correlation coefficient, r, between the residual time courses of each ROI in a pair after regressing out the head-motion parameters and signals from the ventricles and white matter. Structural connectivity between a pair of ROIs was determined using probabilistic tractography to measure the volume, V, of fiber bundles connecting both regions of interest. Both r and V for each ROI pair were evaluated as a function of vision loss and the extent and strength of tactile-evoked activity in V1. Tactile-evoked V1 activities were found to be negatively correlated with resting-state connectivity and fiber volume between the inferior parietal lobe ROI and ROIs in V5, middle temporal area (MT), lateral occipital complex (LOC), and inferior temporal area (IT). Our preliminary findings suggest that tactile-evoked activity in V1 is related to changes in resting-state connectivity and fiber volume between the inferior parietal cortex and other regions of the visual stream (including areas V5, MT, LOC, and IT). This result suggests that changes in parietal-visual association areas are related to tactile processing in the blind.

Disclosures: S.I. Cunningham: None. **J.D. Weiland:** None. **P. Bao:** None. **B.S. Tjan:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.11/RR11

Topic: D.03. Multisensory

Title: Cross-sensory phase reset impacts coherence between sensory and motor cortices: An electro-corticographic study

Authors: *M. R. MERCIER[1,2], M. SOPHIE[1], I. C. FIEBELKORN[1,3], J. J. BUTLER[1], T. H. SCHWARTZ[2], J. J. FOXE[1];
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Bronx, NY; [2]Neurolog. Surgery, Weill Cornell Med. Col., New York, NY; [3]Psychology, Neurosci. of Attention & Perception Lab., Princeton Univ., Princeton, NJ

Abstract: It is generally accepted that brain oscillatory activity reflects fluctuations of neuronal excitability. Different phases of an oscillation correspond to high and low excitatory states. Thus synchronized activity can lead to temporal windows of communication between brain regions involved in a given task (Fries, 2005). It is also the case that the phase of ongoing oscillations can modulate processing of incoming information. "Cross-sensory" stimulation can impact activity in a unisensory cortical region (e.g., visual inputs modulating oscillatory activity in auditory cortex) by resetting the phase of ongoing oscillations (Lakatos et al., 2007 ; Mercier et al., 2013). It has been hypothesized that such early multisensory integration leads to observed speeded reaction-times (Molholm et al., 2002). Here we sought to test for a relationship between this cross-sensory phase resetting mechanism and communication between sensory and motor cortices following multisensory stimulation.

Using intracranial recordings in epileptic patients (n=4), subdural electrical brain activity was recorded while patients performed a simple reaction time task. Stimuli consisted of randomly ordered Auditory-alone (A), Visual-alone (V) and Audio-Visual stimuli (AV). Participants were to press the same button in response to any stimulus, regardless of sensory modality.

For unisensory conditions, analyses revealed cross-sensory phase reset of lower frequencies (δ and θ bands ; 3-8Hz) in unisensory cortices, with auditory-driven phase reset over visual cortex, and visual-driven phase reset over auditory cortex. For the audio-visual condition, stronger phase resetting was observed in both auditory and visual cortex compared to unisensory conditions. Focusing on communication between sensory and motor cortices, significant coherence was observed for the same low frequency bands. The audio-visual condition lead to stronger coherence compared to the uni-sensory conditions, whether considering coherence between auditory and motor or visual and motor regions.

These data support the idea that cross-sensory phase reset plays a role in multisensory interaction in sensory cortices, and that this cross-sensory phase reset can lead to increased synchronization between sensory cortices and motor cortex in the context of a multisensory reaction time task. In conclusion, our study illustrates how neuronal populations from anatomically segregated brain regions can impact each other by resetting the phase of ongoing activity and how multisensory interactions can facilitate communication between sensory and motor cortex through increased coherence.

Disclosures: M.R. Mercier: None. **M. Sophie:** None. **I.C. Fiebelkorn:** None. **J.J. Butler:** None. **T.H. Schwartz:** None. **J.J. Foxe:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.12/RR12

Topic: D.03. Multisensory

Support: KAKENHI 23700632

Title: Number of temporally accurate steps during dance video game correlates with the activity in the middle temporal gyrus and the frontopolar cortex

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Abstract: We used near-infrared spectroscopy to investigate how multimodal sensory integration influences coordinated motor outputs when subjects were able to move freely as they would in the real world. Subjects played a dance video game which requires integrated processing of visual, audio and tactile inputs and motor outputs in two conditions: with and without music (Audio). We focused on two brain areas, the middle temporal gyrus (MTG), an area of multisensory integration, and the frontopolar cortex (FPC), an area associated with multitask decision making. Subjects (21 male, 5 female; 26.1 +/- 1.7 years) responded by pressing the correct arrow button (up, down, left and right) on a dance mat at the correct time with their foot to play the game. We studied cortical function using a block design with 30s of dance play followed by 30s of rest repeated five times. Gameplay performance was scored by the number of temporally accurate steps, which corresponded to proper arrow button press within +/- 22.5ms of the correct time. All subjects showed a bell-shaped oxyHb waveform in the MTG, in which high-performance players took a longer time to reach to peak amplitude than low-performance players. Elimination of music during gameplay increased the cumulative amount of oxyHb signal in the MTG in high-performance players while it decreased in low-performance players. The oxyHb waveforms in the FPC were different among players; high-performance players showed a small oxyHb increase followed by a steep and sustained decrease during the task period, while low-performance players showed box-car shaped with prolonged activation. In spite of the presence or absence of music, the cumulative amount of oxyHb signal in the FPC had a negative correlation with the number of temporally accurate steps. In addition high-performance players had little difference in the oxyHb response in the FPC between the two conditions (with and without audio), while low-performance players showed greater activity during the condition that they perceived to be more difficult. These results suggest that the MTG plays a role in the successful integration of visual and rhythmic cues and that high-performance players are able to integrate visual and internally-generated rhythm to make accurate steps even without external auditory rhythmic cues. The FPC is involved in processing prospective memory while multitasking and may work to compensate for insufficient integrative ability of visual and

rhythmic cues in the MTG in low-performance players. The relative relationships between these cortical areas may indicate high to low performance levels when performing cued motor tasks.

Disclosures: Y. Nomoto: None. **J.A. Noah:** None. **A. Tachibana:** None. **S. Bronner:** None. **S. Shimada:** None. **Y. Ono:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.13/RR13

Topic: D.03. Multisensory

Support: FRQS

NSERC

Title: Enhanced multisensory processing in musicians

Authors: *J. ROY[1,2,3], S. LANDRY[1,2,3], J. LÉVESQUE[2,3], F. CHAMPOUX[1,2,3];
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Abstract: Recent investigations have revealed that long-term musical training promotes brain plasticity and generates reorganization in regions affecting multisensory processing. In addition to these anatomical and activation changes, recent studies have also shown behavioural alterations in multisensory processing induced by long-term musical training. While past investigations of multisensory integration have suggested an enhanced ability, only audiovisual modalities have been studied as of yet. The aim of this study was to examine whether musical training enhanced multisensory integration and segregation abilities for other modalities, namely audiotactile. Two non-speech audiotactile illusory tasks were administered to a group of highly trained musicians and a group of non-musicians. Control conditions revealed that unisensory (detection and discrimination) capabilities were identical across groups. For the first task, musicians were able to segregate auditory and tactile information effectively in the context of an audiotactile illusion, whereas non-musicians were not. For the second task, unlike non-musicians, musicians did not experience any illusory change of tactile perception in presence of modified auditory stimulations. Results from these investigations reveal that musicians have reduced susceptibility to auditotactile illusions, suggesting that auditory and tactile information can be processed more independently in musicians than in non-musicians. These results imply

that long-term musical training has an influence on multisensory processing.
This research was supported by the FRQS and NSERC.

Disclosures: J. Roy: None. **S. Landry:** None. **J. Lévesque:** None. **F. Champoux:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.14/RR14

Topic: D.03. Multisensory

Support: Radboud University Medical Centre, RG000457

Title: Structural brain differences associated with sensory profiles

Authors: *M. T. VAN KESTEREN[1,2], M. R. VAN SCHOUWENBURG[1,3], D. R. RUITER[2], G. FERNÁNDEZ[1,4];
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Abstract: The adult sensory profile questionnaire (Brown et al., 2000) is widely used to assess sensory processing alterations in patient populations with difficulties in this domain, e.g. patients suffering from autism or schizophrenia. However, not much is known about the brain regions related to sensory processing differences in the healthy population, while assessing individual variations in the size of these associated regions could be highly informative as a reference database for comparison with data from groups of patients. Therefore, we here examined structural brain differences related to scores on the adult sensory profile questionnaire in a large healthy female student population (n = 90) to determine brain regions implicated in sensory processing differences. All four factors of the questionnaire, low registration, sensation seeking, sensory sensitivity, and sensation avoiding, were separately examined in relation to grey matter volume as calculated with Voxel-Based Morphometry (VBM) methods on structural brain scans obtained with functional Magnetic Resonance Imaging (fMRI). Results show that higher scores on low registration, sensation avoiding, and sensory sensitivity were related to larger grey matter volume in the bilateral parahippocampal gyrus and extending into the fusiform gyrus. Additionally, higher scores on sensory sensitivity were also related to larger grey matter volume in the bilateral inferior parietal sulcus, and higher scores on sensation seeking were related to larger grey matter volume in the right insula. Finally, lower scores on low registration, thus representing high registration abilities, were related to larger grey matter volume in the thalamus. These results are indicative of grey matter differences in diverse sensory brain regions as related

to sensory profile scores in healthy young participants. These findings therefore provide important reference data on the differential volumes of specific brain regions related to sensory profiles in the healthy population, thereby serving the quest to determine brain regions and networks that are malfunctioning in patient populations. The brain regions found in this experiment can thus be used to focus future research on sensory processing mechanisms within these regions, both in healthy participants and in patients.

Reference

Brown, C., Tollefson, N., Dunn, W., Cromwell, R., & Filion, D. (2001). The Adult Sensory Profile: Measuring patterns of sensory processing. American Journal of Occupational Therapy, 55, 75-82.

Disclosures: M.T. Van Kesteren: None. **M.R. van Schouwenburg:** None. **D.R. Ruiter:** None. **G. Fernández:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.15/RR15

Topic: D.03. Multisensory

Support: NIH Grant 1RO1GM098578

Title: Reconfiguration of network hub structure after propofol-induced unconsciousness

Authors: U. LEE[1], H. LEE[2], *G. A. MASHOUR[1];
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Abstract: Introduction: General anesthesia induces unconsciousness along with functional changes in brain networks. Considering the essential role of hub structures for efficient information transmission, we hypothesized that anesthetics have an effect on the hub structure of functional brain networks.
Methods: Graph theoretic network analysis was carried out to study the network properties of 21-channel electroencephalogram data from ten human volunteers anesthetized on two occasions. The functional brain network was defined by phase lag index, a coherence measure, for three states: wakefulness, loss of consciousness induced by the anesthetic propofol, and recovery of consciousness. The hub nodes were determined by the largest centralities. The correlation between the altered hub organization and the phase relationship between electroencephalographic channels was investigated.

Results: Topology rather than connection strength of functional networks correlated with states of consciousness. The average path length, clustering coefficient and modularity significantly increased after propofol administration, which disrupted long range connections. In particular, the strength of hub nodes significantly decreased. The primary hub location shifted from the parietal to frontal region in association with propofol-induced unconsciousness. The phase lead of frontal to parietal regions in the alpha frequency band (8-13 Hz) observed during wakefulness reversed direction after propofol and returned during recovery.
Conclusions: Propofol reconfigures network hub structure in the brain and reverses the phase relationship between frontal and parietal regions. Changes in network topology are more closely associated with states of consciousness than connectivity and may be the primary mechanism for the observed loss of frontal-to-parietal feedback during general anesthesia.
Acknowledgments: Funding support from Dept. Of Anesthesiology and NIH RO1 (GAM), is acknowledged

Disclosures: U. Lee: None. **G.A. Mashour:** None. **H. Lee:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.16/RR16

Topic: D.03. Multisensory

Support: Intramural Research Program of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health

Title: Parietal short intracortical inhibition: parietal double-pulse transcranial magnetic stimulation

Authors: R. W. PAINE[1], A. N. KARABANOV[2], C.-C. CHAO[3], *M. HALLETT[1];
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Abstract: Intracortical circuits in the motor cortex have been analyzed using double pulse transcranial magnetic stimulation (TMS), but these circuits have only rarely been studied in other cortical regions. Previous experiments have shown that transcranial magnetic stimulation (TMS) of parietal cortex may facilitate or inhibit subsequent motor cortical evoked potentials. We investigated whether intraparietal circuits can be affected by double-pulse TMS, thereby altering parietal excitability, influencing the functional connection to the primary motor cortex, and thus

providing a means of assessing the intraparietal circuits.
23 healthy volunteers received TMS while seated at rest. The facilitatory and inhibitory effects on motor cortex by parietal conditioning pulses seen in previous studies were replicated. The subjects also received double-pulse stimulation of the parietal cortex consisting of a pre-conditioning TMS pulse followed by a conditioning pulse, and then a test pulse to the motor cortex. Intervals of 2, 4, and 8 ms between the parietal pre-conditioning and conditioning pulses were tested. There was significant neutralization of parietal-motor cortical facilitation by a parietal pre-conditioning TMS pulse presented 2 ms before the parietal conditioning pulse. There was also a non-significant trend toward neutralization of parietal-motor cortical inhibition by a parietal pre-conditioning TMS pulse presented 2 ms before the parietal conditioning pulse. These results suggest the existence of parietal short intracortical inhibition (pSICI) which is analogous to the short intracortical inhibition (SICI) that has previously been observed in motor cortex.

Disclosures: R.W. Paine: None. **M. Hallett:** None. **A.N. Karabanov:** None. **C. Chao:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.17/RR17

Topic: D.03. Multisensory

Support: LOEWE - Neuronale Koordination Forschungsschwerpunkt Frankfurt (NeFF)

Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme

Title: Neural synchronization during bottom-up and top-down visual processing in grapheme-color synesthetes and schizophrenia patients

Authors: T. M. VAN LEEUWEN[1,2], M. WIBRAL[3], A. SAUER[1,2], P. J. UHLHAAS[1,5], W. SINGER[1,2,4], *L. MELLONI[6,1,2];
[1]Dept. of Neurophysiol., Max Planck Inst. for Brain Res., Frankfurt am Main, Germany; [2]Ernst Strüngmann Inst. (ESI) for Neurosci. in cooperation with Max Planck Society, Frankfurt am Main, Germany; [3]Brain Imaging Ctr., [4]Frankfurt Inst. for Advanced Studies, Johann Wolfgang Goethe Univ., Frankfurt am Main, Germany; [5]Inst. of Neurosci. and Psychology, Univ. of Glasgow, Glasgow, United Kingdom; [6]Exptl. Therapeut., Columbia Univ., New York, NY

Abstract: We used a visual perceptual closure task to investigate the influence of both synesthesia, as a hyper-synchronization model, and of schizophrenia, as a hypo-synchronization

model, on the threshold of conscious perception. In grapheme-color synesthetes, letters and numbers evoke color. Synesthesia may involve hyperbinding of color and graphemes; at the neural level, binding processes are associated with increased synchronization between different features. In schizophrenia patients, neuronal synchronization is impaired and problems with perceptual processing exist.
Twenty synesthetes, 20 controls, and 15 schizophrenia patients viewed letters and numbers that elicited synesthesia for the synesthetes, and non-synaesthetic control stimuli (symbols). Stimuli were embedded in a noise background, which was colored congruently with the synesthetic color (for synesthetes) or neutrally colored (symbols). The amount of noise was parametrically varied and the visibility threshold of the embedded grapheme was determined by subjective visibility ratings. A bottom-up (increasing contrast of the grapheme) as well as a top-down (decreasing contrast) condition was included to assess hysteresis (memory) effects that aid recognition in the top-down condition.
We observed clear differences in the threshold of perception across groups in the bottom-up condition. Schizophrenia patients showed reduced recognition of the stimuli compared to the other groups, for both letters and symbols. Synesthetes in turn showed a clear behavioral advantage with a lower threshold of perception than controls, specific for the synesthesia-inducing condition (letters). Thus, synesthetic hyperbinding seems to aid synesthetes during closure. We observed a clear effect of top-down predictions - a lower perceptual threshold when subjects could anticipate the stimuli. Critically, the perceptual gain was similar in size for all groups, indicating that schizophrenia patients, in our paradigm, do not show impairment in top-down processing.
Time-frequency analyses of magnetoencephalography data showed induced gamma band activity (50-70 Hz and 80-100 Hz) over occipital sensors. For letters, synesthetes showed increased gamma power compared to controls (50-70 Hz). Schizophrenia patients showed less gamma power in the 50-70 Hz band than controls. Source localization (50-70 Hz) of successfully identified graphemes revealed activity in early visual areas (V2/V3) and in color area V4 and parietal cortex. We suggest that altered gamma activity in synesthetes reflects hyperbinding and that bottom-up deficits of perception in schizophrenia are related to gamma band activity.

Disclosures: T.M. van Leeuwen: None. **L. Melloni:** None. **A. Sauer:** None. **W. Singer:** None. **P.J. Uhlhaas:** None. **M. Wibral:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.18/RR18

Topic: D.03. Multisensory

Title: Development of an evaluation method for degrees of synesthetic perception

Authors: *D. NAKAJIMA[1], H. MAZAKI[1], R. YAYAMA[1], K. KATAHIRA[1], A. SHIRAIWA[1], E. AIBA[1,2,3], N. NAGATA[1];
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Abstract: Synesthesia is a perceptual phenomenon in which one sensory stimulus involuntarily induces perception in another sense. Being able to easily distinguish between synesthetes and non-synesthetes would be useful in developing laboratory research on synesthesia. However, many current methods are based on self-reported experience, which is not sufficient for objectively discriminating between synesthetes and
non-synesthetes. Thus, in this study, we have attempted to develop and test a method that discriminates between synesthetes and non-synesthetes and that also evaluates the degree of individuals' synesthetic perception by utilizing a task to which synesthetes and non-synesthetes were expected to respond with different patterns. The test consisted of an evaluation task that measured participants' emotional responses to combinations of letters and colors for grapheme-color synesthesia and combinations of music and color for sound-color synesthesia. We then attempted to develop an objective indicator based on their response patterns. We focused on high repeatability and clarity of evaluation, which seem to be criteria for judging degrees of synesthetic perception that are unique to and typical of synesthetes. Using the task mentioned above, we tested 11 participants. Seven of them were previously identified as synesthetes and four were non-synesthetes. In order to confirm response repeatability, the experiments were conducted twice, and the second experiment was executed at least a week after the first one. In addition, during the second experiment, the order of stimulus presentation was changed compared to the first experiment. As expected, the repeatability of the evaluation of each stimulus was higher in the group of synesthetes than in the non-synesthetes. Furthermore, as the synesthetes showed emotional responses
to each stimulus more clearly, it may be possible to construct a continuous scale discriminating between synesthetes and non-synesthetes based on these two criteria of "repeatability" and "clarity."

Disclosures: D. Nakajima: None. **H. Mazaki:** None. **R. Yayama:** None. **K. Katahira:** None. **A. Shiraiwa:** None. **E. Aiba:** None. **N. Nagata:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.19/RR19

Topic: D.03. Multisensory

Support: Luxembourg Research Counsil (FNR) AFR 2y Postdoc grant awarded to Dr. N. Bien (2011)

Title: Investigating the links between spatial-numerical interactions and sequence processing using patterned TMS

Authors: *N. BIEN[1], A. T. SACK[2], C. SCHILTZ[1];
[1]Univ. of Luxembourg, Walferdange, Luxembourg; [2]Maastricht Univ., Maastricht, Netherlands

Abstract: Number perception often results in response facilitation: small numbers facilitate left hand responses, while large numbers speed up right hand responses (spatial numerical association of response codes; SNARC1). There is currently debate about whether it is the cardinality (magnitude) aspect of numerical processing that causes spatial facilitation2, or rather ordinality aspects associated with working memory coding / sequence coding strategies3,4. SNARC has also been linked to crossmodal assocations and even to spatial sequence synesthesia5,6. The neural bases of the SNARC effect are still elusive, but are mostly hypothesized to be found in parietal and frontal brain regions that are part of the dorsal 'where' processing stream7,8.

Here, we investigated if, and how, magnetic disruption (patterned TMS) of a region in right middle temporal gyrus (rMTG), previously linked to processing of overlearned ordinal sequences9, affects SNARC performance in healthy adults. In addition, we assessed the spatial layout and strength of their numerical mental representations, and correlated it with SNARC performance. Finally, we linked SNARC characteristics to sequence processing performance.

We found a significant SNARC effect in our n=30 sample ($p = 0.0001$). After inhibitive patterned TMS over sequence processing region rMTG, this SNARC effect was significantly reduced ($p = 0.027$), confirming the relevance of ordinal sequence information retrieval for spatial-numerical interactions.

However, TMS preferentially affected participants with strong left-to-right mental-numerical representations ($p=0.048$). This implies that sequence information processed in the rMTG especially contributes to spatial-numerical associations in people with stronger left-to-right mental numerical representations.

Finally, we found that whereas TMS over rMTG in general impairs sequence processing performance, it actually improves the below-average performance of the subgroup of our sample that displays the strongest SNARC effect: they perform below average on sequence processing, but improve to a normal level after disruption of the rMTG sequence processing area.

In conclusion, our data provide important new input to ongoing debates about spatial-numerical processing, especially considering the cardinal vs. ordinal nature of the effect, and individual

differences in spatial-numerical associations.
1. Dehaene, 1993
2. Walsh, 2003
3. Van Dijck & Fias, 2011
4. Lindemann et al., 2008
5. Cohen Kadosh & Henik, 2007
6. Eagleman, 2009
7. Rusconi et al., 2011
8. Rusconi et al., 2007
9. Pariyadath, 2008

Disclosures: N. Bien: None. **A.T. Sack:** None. **C. Schiltz:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.20/RR20

Topic: D.03. Multisensory

Support: Senescyt Grant for the Author supported by the Ecuatorian Goverment

Title: Gender differences in cortico-subcortial network coupling during facial expressions

Authors: *F. R. LOAYZA[1,2], J. BUENDÍA[3], M. CELORRIO[2], F. VILLAGRA[2], B. HONTANILLA[3], M. PASTOR[2,3];
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Abstract: Objective: To investigate the neural mechanism underlaying facial expressions and gender differences observed during post-surgery facial paralysis rehabilitation, using fMRI.
Background: Facial paralysis rehabilitation is influenced by several factors mainly dependent of denervation time and muscle atrophy. It has also been reported that gender plays an important role in rehabilitation, being women whose perform better spontaneous facial expressions than men after surgery. Facial expressions are mainly controlled by two neuronal networks: the involuntary “emotionally driven” and the voluntary. However, little is known about the relationship between them, and moreover whether these are gender dependent.
Methods: We recruited 24 healthy volunteers (11F) to perform an fMRI experiment. The paradigm included two visually presented tasks: smile, chew and a fixation cross. For the motor

tasks, subjects were instructed to perform a slight smile/chew face movement minimizing jaw movements. Facial kinematics were also measured (CLIMA System). Imaging data were processed using SPM8-Dartel. At the second level, we used 2-way ANOVA with factors gender and task. Psichophysiological Interactions analyses (PPI) were used to evaluate connectivity of the key pre-motor regions during both tasks. A 2-way ANOVA was also used to compare PPI results.
Results: Kinematics of facial movements did not show any significant gender differences. The imaging results showed similar brain activity between male and female subjects during facial movements without significant differences between them. However, PPI results showed significant gender differences: The main effect of gender showed differences in the interaction of bilateral facial premotor region with bilateral anterior cingulate cortex including pre-SMA, amygdala and hippocampus, and to the thalami, including prefrontal, motor and premotor thalamic regions.
Conclusions: Our findings suggest that the degree of coupling of a cortico-subcortical network mediating simple facial expressions may be gender dependent.

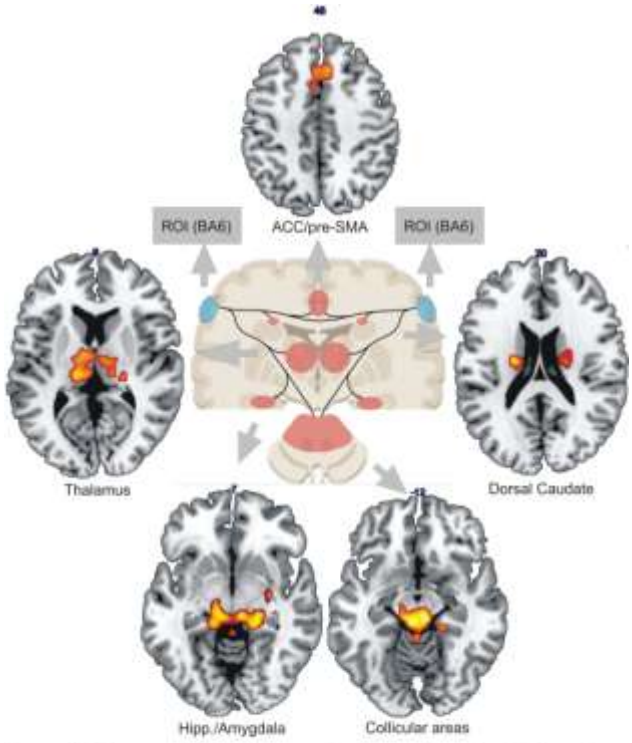

Image depicts connectivity changes in the cortico-subcortical pathway related to gender differences during facial expressions p<0.001 FDR cluster corrected

Disclosures: **F.R. Loayza:** A. Employment/Salary (full or part-time):; 3800. **J. Buendía:** None. **M. Celorrio:** None. **F. Villagra:** None. **B. Hontanilla:** None. **M. Pastor:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.21/RR21

Topic: D.14. Cerebellum

Support: The Johns Hopkins University Brain Science Institute

NIH/NICHD Grant P30HD024061

NIH/NINDS Grant F32NS073371

Title: Direct comparison of network connectivity revealed by resting-state fMRI and concurrent TMS-fMRI

Authors: *J. M. YAU[1], M. B. NEBEL[3], J. HUA[2], J. E. DESMOND[1];
[1]Neurol., Johns Hopkins Univ., BALTIMORE, MD; [2]Russell H. Morgan Dept. of Radiology and Radiological Sci., Johns Hopkins Univ., Baltimore, MD; [3]Kennedy Krieger Inst., Baltimore, MD

Abstract: Brain regions collaborate in distributed networks. With resting-state functional connectivity analyses, network architecture is inferred from spatially distributed, coherent low-frequency BOLD fluctuations. Resting-state connectivity patterns tend to conform to anatomical connectivity maps and there is a high correspondence between functional connectivity patterns and task-evoked coactivation patterns revealed by conventional functional neuroimaging. Despite these demonstrations, the direct relationship between correlated spontaneous BOLD fluctuations and acute neural processing remains untested in healthy adults. Because transcranial magnetic stimulation (TMS) can be used to causally evoke neural responses in targeted brain regions and in remote but connected areas, this research tool offers a unique opportunity, when paired with fMRI, to relate rs-fMRI network analysis and dynamic neural processes.
Here, we demonstrate that TMS-evoked BOLD activation patterns closely relate, but are not identical, to resting-state functional connectivity. During each scan session, we first acquired rs-fMRI data from participants, prior to TMS administration in the scanner. While participants remained at rest in concurrent TMS-fMRI scans, we causally evoked distinct and robust BOLD activation patterns with targeted brain stimulation. Using TMS target locations as region-of-interest seeds, we computed seed-based correlation maps with a separate rs-fMRI dataset (Kirby 21; http://mri.kennedykrieger.org/databases.html). Defining networks using this large and independent dataset enabled us to generate robust statistical masks that we used to query and compare each participant's rs-fMRI data and concurrent TMS-fMRI data. We found substantial overlap in TMS-evoked BOLD activation patterns and seed-based functional connectivity, providing evidence for the hypothesis that resting-state BOLD fluctuations reflect intrinsic network architecture that supports acute and dynamic neural processing. However, TMS also

evoked responses that were distinct from seed-based connectivity patterns indicating that concurrent TMS-fMRI may reveal additional network relationships.

Disclosures: J.M. Yau: None. **M.B. Nebel:** None. **J. Hua:** None. **J.E. Desmond:** None.

Poster

550. **Mutisensory: Cross-Modal Processing in Humans**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 550.22/RR22

Topic: D.03. Multisensory

Support: All authors are supported by grants from NASA and the NSBRI.

Title: Resting state functional connectivity before, during and after 70 days of bed rest

Authors: *B. ERDENIZ[1], V. KOPPELMANS[1], J. BLOOMBERG[2], Y. E. DE DIOS[3], I. KOFMAN[3], D. SZECSY[3], M. FIEDLER[3], B. PETERS[3], E. ALLEN[3], R. RIASCOS-CASTANEDA[4], A. P. MULAVARA[5], R. SEIDLER[1];
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Abstract: The bed rest protocol is designed by NASA and offers researchers from a variety of backgrounds the possibility to study bed rest as an experimental analog for space flight. It has been shown that extended exposure to a head-down tilt position can resemble many of the effects of a low-gravity environment (Seaton et al., 2009). Here, we use a head-down tilt bed rest intervention to investigate the neural and neurocognitive effects of extended duration reduced sensory inputs and increased cephalic fluid distribution (Koppelmans et al., 2013). Thus far, complete data including resting state MRI of two participants have been acquired through the bed rest facility located at the University of Texas Medical Branch (Galveston). Subjects remained in bed with their heads tilted down 6 degrees below their feet for 70 consecutive days. Behavioral measures and neuroimaging assessments were obtained at seven time points: a) two measurements took place approximately 7 and 12 days before bed rest; b) three measurements took place at approximately 7, 30, and 65 days during bed rest; and c) two measurements took place at approximately 7 and 12 days after bed rest. Functional connectivity magnetic resonance imaging (FcMRI) analysis was performed to assess the connectivity between eighteen regions of interest (ROI). Based on a prior study (Van Dijk et al., 2009) six of the ROIs were identified to be in the default mode network, six ROIs were identified to be in the attention network, and the remaining six were identified to be in the motor, visual and auditory cortex. The connectivity

matrix of the ROI regions was analyzed using graph theory for all seven time points (Rubinov and Sporns, 2010). We report that during the first week of bed rest, connectivity between regions strengthened throughout the brain, and then gradually returned to baseline levels as the bed rest period continued and remained at baseline levels during post bed rest testing.

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Disclosures: B. Erdeniz: None. **V. Koppelmans:** None. **R. Seidler:** None. **J. Bloomberg:** None. **Y.E. De Dios:** None. **I. Kofman:** None. **D. Szecsy:** None. **M. Fiedler:** None. **B. Peters:** None. **E. Allen:** None. **R. Riascos-Castaneda:** None. **A.P. Mulavara:** None.**Poster**

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.01/RR23

Topic: D.03. Multisensory

Support: NSF Graduate Research Fellowship

T. H. Ashford Graduate Fellowship

Title: Multisensory processing in the zebrafish escape circuit

Authors: *A. M. LACOSTE, D. SCHOPPIK, D. N. ROBSON, J. M. LI, F. ENGERT, A. F. SCHIER;
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Abstract: Animals use multiple sensory systems to create an accurate perception of the world and to inform behavioral decisions. Understanding how neural circuits responsible for behavior integrate multisensory cues from the environment is a challenge due to the complexity of the networks involved. The large number of neurons in most vertebrate preparations necessitates a statistical approach to sensory representation and downstream decoding. By contrast, the Mauthner neuron-mediated zebrafish escape circuit is small and well-characterized. Yet it is a

sophisticated network responsible for a behavioral decision essential to the animal's survival. This circuit allows us to answer questions of neural computations in a transparent, behaving brain, at the level of individual genetically accessible neurons. We are investigating how a previously uncharacterized excitatory interneuron in the circuit encodes different types of stimuli to influence escapes.

Disclosures: A.M. Lacoste: None. **D. Schoppik:** None. **D.N. Robson:** None. **J.M. Li:** None. **F. Engert:** None. **A.F. Schier:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.02/RR24

Topic: D.03. Multisensory

Support: DFG

Title: A thalamic reticular nucleus circuit that regulates the selection of sensory inputs

Authors: *S. AHRENS[1], S. JARAMILLO[1], S. GHOSH[1], C. LAI[2], J. Z. HUANG[1], B. LI[1]; [1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Idiana Univ., Bloomington, IN

Abstract: Selective processing of some sensory stimuli at the expense of others is a fundamental brain function. The thalamic reticular nucleus (TRN) is thought to gate the transmission of information en route to the cortex. TRN neurons, which are exclusively GABAergic, receive glutamatergic inputs from the cortex and thalamus, and send projections to the thalamus where they directly modulate the activity of thalamic relay neurons. However, its role in selecting behaviorally relevant sensory cues is unclear. Here, we probed and manipulated the somatostatin (SOM+) class of neurons in the TRN, which highly express ErbB4, a gene that is involved in controlling glutamatergic synapse maturation and function and linked to psychiatric disorders such as schizophrenia. We have generated SOM/ErbB4-/- mice in which ErbB4 gene is ablated in SOM+ neurons in the TRN. Using ChR2 to selectively stimulate the cortical or thalamic input onto TRN neurons in brain slices, we found that the absence of only one copy of ErbB4 in SOM+ TRN neurons is sufficient to alter cortico-thalamic (CT) transmission but without affecting thalamo-cortical (TC) transmission. As a consequence the feed-forward inhibition of TRN neurons onto thalamic relay neurons driven by the CT-pathway is also altered. Moreover, we found that ErbB4 deficiency in SOM+ TRN neurons alters the performance of mice in behavioral tasks engaging competition of either within-modality or across-modalities sensory inputs. Our results indicate that the SOM+ TRN neurons are required for the selection of

salient sensory information, and furthermore reveal an unexpected role of ErbB4 in this process by controlling specific synapses in the cortico-TRN-thalamic circuitry.

Disclosures: S. Ahrens: None. **S. Jaramillo:** None. **S. Ghosh:** None. **C. Lai:** None. **J.Z. Huang:** None. **B. Li:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.03/RR25

Topic: D.03. Multisensory

Support: NIH Grant R01-EY022720

NIH Grant F31-NS079058

Title: Potentiation of feed-forward inputs in auditory cortex after visual deprivation

Authors: *E. R. PETRUS[1], H.-K. LEE[2];
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Abstract: The loss of vision causes sensory compensation in spared sensory modalities. For example, blind individuals experience enhanced performance on auditory discrimination tasks, such as tone localization (Roder et al. 1999) and pitch discrimination (Gougoux et al. 2004). The underlying synaptic changes associated with these cross-modal adaptation were first observed as global changes in the strength of excitatory synaptic inputs, which was measured as alterations in miniature excitatory post synaptic current (mEPSC) amplitudes, in visually deprived mice (Goel et al. 2006). mEPSC amplitude changes occurred in primary visual cortex (V1), with inverse changes in somatosensory (S1) and auditory (A1) cortices after seven days of dark exposure (DE). In A1, mEPSC amplitudes change in a lamina specific manner following DE, with a potentiation seen in layer 4 (L4) neurons, and a reduction in layer 2/3 (L2/3) neurons. The potentiation of inputs to L4 neurons in A1 was due to a stronger thalamocortical input. Furthermore, DE resulted in a stronger feed-forward input to L2/3 from L4. Lateral inputs to L4 were reduced in strength, which indicates an increase in the strength of feed-forward signal to lateral input ratio in A1 after DE. These changes in synaptic weights may be a mechanism underlying the enhancement of auditory performance in blind individuals.

Disclosures: E.R. Petrus: None. **H. Lee:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.04/RR26

Topic: D.03. Multisensory

Support: NSC Grant 101-2321-B-002-078, National Science Council of Taiwan

Title: Auditory signals affect the responses of neurons in early visual stages of awake rats

Authors: *C.-I. YEH[1,2,3], K.-S. LEE[1,2];
[1]Psychology, [2]Neurobio. and Cognitive Sci. Ctr., [3]Grad. Inst. of Brain and Mind Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Detecting changes is a critical feature of sensory processing. This ability can be enhanced by integrating information from different sensory modalities in natural environment. In the visual system, light increments and decrements are processed through parallel ON and OFF channels from the retina to the lateral geniculate nucleus (LGN). The ON and OFF channels then converge for the first time in the primary visual cortex (V1). Similarly in the auditory system, UP (stimulus onset) and DOWN (stimulus offset) signals remain segregated before converging in the primary auditory cortex (A1) (He, J. 2001, Scholl et al., 2010). A recent study has shown that the visual responses of most V1 neurons are greatly suppressed by auditory signals in rodents (Iurilli et al., 2012). However, it is unclear whether auditory signals may modulate V1 ON and OFF channels differently or the audio-visual modulation may depend on the degree of congruence (e.g. UP-ON versus DOWN-ON). We addressed these questions by recording from 82 V1 units and 30 LGN units in awake Long-Evans rats. Brief auditory and visual stimuli (200 ms) were presented simultaneously, and a total of 25 bimodal conditions (5x5) were used (auditory stimuli: mean: 80 dB; UP/DOWN: +13% and +25%; visual stimuli: mean: 70cd/m2; ON/OFF: +35% and +70%). Preliminary results showed that the effect of the bimodal stimulation was predominantly sub-additive for neurons in both regions - the bimodal responses were weaker than the linear combination of the two excitatory unimodal responses. Interestingly, the sub-additive bimodal response to the OFF-DOWN pair in V1 was significantly lower ($P<0.05$) than the responses under other visuo-auditory stimulus pairs. In contrast, there is no significant difference for LGN neurons among the four pairwise conditions, though the response tendencies in LGN and V1 were very similar. Overall, these results suggest that a linear combination of auditory and visual responses cannot explain the complex interaction between ON/OFF channels and UP/DOWN channels. We are applying a weighted linear model with various combinations of auditory UP/DOWN and visual ON/OFF inputs to search for possible mechanisms.

Disclosures: C. Yeh: None. **K. Lee:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.05/SS1

Topic: D.03. Multisensory

Support: EU Grant ERC-AdG-269716

DFG Grant SFB 936/A2

Title: Early multisensory interactions revealed through simultaneous recordings from superior and inferior colliculus in the ferret

Authors: I. STITT, E. GALINDO-LEON, F. PIEPER, K. J. HOLLENSTEINER, G. ENGLER, *A. K. ENGEL;
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Abstract: The mammalian superior (SC) and inferior colliculi (IC) are highly conserved midbrain structures that are located very early along the visual and auditory processing pathways, respectively. Although the IC is thought to be primarily an auditory structure, it does receive inputs from multiple sub-cortical and cortical non-auditory structures, such as the SC. Indeed, previous studies in the barn owl have shown that the external nucleus of the IC responds to visual stimulation following pharmacological disinhibition in the avian equivalent of the SC, the optic tectum. However, the multisensory interplay between these two midbrain structures remains poorly understood in mammals. We recorded multi neuron activity (MUA) and local field potentials (LFP) from the SC and IC simultaneously with silicon multichannel probes in the anaesthetised ferret. Visual stimuli consisted of flashes and drifting gratings, while auditory stimuli consisted of clicks and pure tones. To assess multisensory effects, flashes and clicks were presented with stimulus onset asynchronies from 0 to 200ms in 10ms steps. Although flash stimuli failed to evoke spiking activity in IC neurons, we consistently observed a visually-evoked concentration of LFP oscillatory phase in the 6-10Hz and 15-30Hz frequency bands. Interestingly, phase-locking was not accompanied by an increase in oscillatory power, suggesting that visual stimuli only modulate the phase of ongoing oscillations. To determine the temporal order of flash-evoked phase-locking between midbrain structures, we computed the phase slope index (PSI) between all SC and IC probe contacts. Flash-evoked oscillatory phase changes (15-30Hz band) occur first in the superficial SC, followed by the IC, and then later by the deep layers of the SC. This suggests the superficial layers of the SC as a possible source of phase modulation in the IC. We found several cases in which MUA responses to clicks in the IC

were significantly modulated by preceding flash stimuli. In such cases, click responses were either enhanced or suppressed at specific stimulus onset asynchronies, suggesting flash-evoked oscillatory phase-locking may modulate auditory responses. Previous studies in the auditory cortex have observed similar modulation of auditory responses by somatosensory or visual stimuli. These authors suggest that resetting oscillatory phases might bias local networks towards high or low states of excitability, amplifying or suppressing responses to subsequent auditory inputs. Our data indicate that similar mechanism may be operating in the ferret IC.

Disclosures: I. Stitt: None. **A.K. Engel:** None. **E. Galindo-Leon:** None. **F. Pieper:** None. **K.J. Hollensteiner:** None. **G. Engler:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.06/SS2

Topic: D.03. Multisensory

Support: NIH Grant DC004845

Title: Responses of prefrontal multisensory neurons to mismatching faces and vocalizations

Authors: *M. M. DIEHL[1], M. D. DILTZ[2], L. M. ROMANSKI[2];
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Abstract:
Evidence has suggested that the multisensory integration of social communication information enhances perception compared to the processing of unimodal stimuli. The ventral frontal lobes are one of several brain regions involved in processing social communication information including faces and vocalizations. We have previously demonstrated that single neurons in ventrolateral prefrontal cortex (VLPFC) respond to and integrate conspecific vocalizations and their accompanying facial gestures (Sugihara, et al, 2006; Diehl and Romanski, 2011). We were therefore interested in how VLPFC neurons responded differentially to matching (congruent) versus mismatching (incongruent) facial and vocal stimuli. We recorded VLPFC neurons during the presentation of movies with congruent or incongruent species-specific facial gestures and vocalizations as well as their unimodal components. Recordings showed that a subset of multisensory VLPFC neurons exhibited a significant change in neuronal activity during incongruent vocalization movies. Furthermore, the majority of these incongruent-responsive cells exhibited incongruent suppression during the early phase of the stimulus period, whereas

other incongruent-responsive neurons exhibited incongruent enhancement in the late phase of the stimulus period when compared to the neuronal responses for congruent audiovisual stimuli. These results suggest that ventral prefrontal neurons are sensitive to differences between congruent and incongruent face-vocalization stimuli that may be important in identity processing or more generally, recognition, and confirms the role of VLPFC in the processing and integration of multisensory communication information.

Disclosures: M.M. Diehl: None. **M.D. Diltz:** None. **L.M. Romanski:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.07/SS3

Topic: D.03. Multisensory

Support: FNS-132465

Title: Multisensory integration in non-human primates during a sensory-motor task

Authors: *F. LANZ, V. MORET, E. M. ROUILLER, G. LOQUET;
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Abstract: In everyday life our central nervous system receives signals via several sensory modalities, processes them and integrates information in order to finally produce an appropriate behaviour. The amazing part is that such a multisensory integration brings all information into a unified percept. A common approach to start investigating this property is to show that discrimination is better and faster when multimodal stimuli are used as compared to unimodal stimuli. This is what we did in our non-human primates' model (n=2) engaged in a detection sensory-motor task where visual and auditory stimuli were displayed individually or simultaneously. Our protocol allowed us to extract several types of behavioural data at every trial: a reaction time (RT) between the percept and the arm movement, a percentage of successes and errors, and the evolution as a function of time of these data to describe the impact of progressive training. As expected, the results showed shorter RTs when the subjects got combined stimuli. The gains were approximately 20msec, as compared with the auditory stimulus alone and 40msec as compared with the visual stimulus alone. These gains were similar for both subjects. In addition the number of correct responses increased when combined stimuli were delivered. The successes and errors percentages demonstrated that subjects are mainly doing execution errors rather than detection errors. We discussed such a multisensory advantage

through redundant signal effect (RSE) which decreases perceptual ambiguity, increases the speed of stimulus detection and improves the preciseness of a performance (RT facilitation).
To get a better understanding of how visual and auditory inputs are combined to generate a unified perception we collected electrophysiological data during behavioural sessions. Single units were derived from the premotor cortex and compared to previous recordings from our laboratory in another polysensory area: the temporal lobe. The results showed that some neurons presented a modulation during a period corresponding to the RTs. In response to an individual stimulus (visual or acoustic) these modulations consisted in inhibitory responses, temporal modifications, delays or an increase of activity. When two stimuli were simultaneously presented, some other neurons responded with either more spikes discharges or a total inhibition. These preliminary steps towards the ambitious goal of gaining access to the knowledge of multisensory integration could help as well to better understand still undiscovered mechanisms of some neurological disesases.

Disclosures: F. Lanz: None. **V. Moret:** None. **E.M. Rouiller:** None. **G. Loquet:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.08/SS4

Topic: D.03. Multisensory

Support: W911NF-08-1-0216

NIH EB00856

NWO VICI grant to P.D.W. (#453-04-002)

NWO VENI grant to M.R. (#451-09-025)

Title: Predictive modulation of alpha and gamma activity and their interaction during auditory and motor tasks in monkey and human

Authors: *A. DE PESTERS[1,2], P. BRUNNER[1,3,4], A. GUNDUZ[5], A. RITACCIO[3], C. MEHRING[6], P. DE WEERD[7,8], M. ROBERTS[8], N. BRUNET[8], R. OOSTENVELD[8], P. FRIES[8,9], G. SCHALK[1,2,3];
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Cognitive Neurosci., Fac. of Psychology and Neuroscience, Maastricht Univ., Maastricht, Netherlands; [8]Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands; [9]Ernst Strügmann Inst. for Neurosci., Frankfurt, Germany

Abstract: Current understanding of cortical rhythms and their relationship to asynchronous local activity during sensory and motor processing is still incomplete. Previous studies suggested that variations in oscillatory activity in the alpha band (8-12 Hz) may gate information between task-relevant cortical areas by inhibiting activity in task-irrelevant areas (Klimesch 2007, Jensen 2010). Thus, modulation of alpha activity may be predictive of relative engagement or disengagement of different cortical systems during different tasks. However, the relationship of such modulatory alpha activity to population-level cortical activity in the high gamma range (70-170 Hz), and the relationship between these neural signals across different tasks, remains undefined. Our ongoing study is investigating the temporal and spatial dynamics of the modulations in the gamma and alpha-bands during auditory and motor tasks. We recorded electrocorticographic (ECoG) activity from subdural electrode grids in five human subjects with epilepsy and one macaque monkey during the presentation of natural auditory stimuli and a simple motor task. Our results confirm that gamma activity accurately tracks task-related behavior and demonstrate that alpha activity is predictively suppressed in task-relevant areas, and increased in task-irrelevant areas, respectively (i.e., for auditory stimulation, alpha suppression in auditory areas and alpha increase in motor areas). Our findings also demonstrate that in the auditory system, alpha suppression trails gamma activity, but precedes gamma activity in the motor system. This finding prompts the hypothesis that cortical excitability may be a result of sensory stimulation, but a facilitator of motor movements. We expect that with additional refinements in our analyses, this work will contribute to our understanding of general mechanisms of the coordination of auditory and motor function in the brain.

Disclosures: A. De Pesters: None. **P. Brunner:** None. **A. Gunduz:** None. **A. Ritaccio:** None. **C. Mehring:** None. **P. de Weerd:** None. **M. Roberts:** None. **N. Brunet:** None. **R. Oostenveld:** None. **P. Fries:** None. **G. Schalk:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.09/SS5

Topic: D.03. Multisensory

Support: NINDS NS035103 to LK

NINDS NS16446 to JHK

Title: Corticocortical connections of area 5 in macaque monkeys support the existence of functionally distinct medial and lateral regions

Authors: *D. F. COOKE[1,2], J. PADBERG[3], C. M. CERKEVICH[4], J. H. KAAS[4], L. KRUBITZER[1,2];
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Abstract: The rostral bank of the macaque intraparietal sulcus (IPS) and the adjacent gyral surface caudal to area 2 constitutes the original Brodmann Area 5 (BA5). This region in posterior parietal cortex (PPC) has been divided into multiple cortical fields with sometimes-conflicting schemes of organization, but all evidence points to a sensorimotor function of BA5 related to reaching and grasping. We term the rostrolateral portion of BA5 lateral area 5 (5L), distinguishing it from surrounding cortex based on its functional organization and cortical architecture. The medial portion of BA5 overlaps the medial intraparietal area (MIP) and the functionally-defined parietal reach region (PRR). To further distinguish medial and lateral portions of area 5 we examined corticocortical connections of area 5 in macaque monkeys. Retrograde tracers (cholera toxin B, Fluoro-ruby, Fast Blue) were injected into electrophysiologically-identified portions of the hand representation in both lateral and medial portions of BA5. Resulting connectional data were related to histologically identified boundaries. We observed two distinct patterns of connections for lateral versus medial portions of BA5. In addition to dense intrinsic connections, 5L received dense connections from portions of areas 3a, 3b, 1 and 2 that correspond to the representation of the forelimb, from similar locations in M1, and from medial portions of BA5. Sparse to moderate connections were also observed from S2, areas 7b, 7a, premotor cortex, cortex medial to area 5 and the supplementary motor area (SMA) extending onto the medial wall.
In sharp contrast, medial BA5 received little or no input from anterior parietal cortex, M1, S2, 7b, or lateral area 5. Instead, it received dense connections from SMA, large portions of prefrontal cortex, the lateral portion of premotor cortex and from anterior cingulate cortex on the medial wall.
Our results demonstrate that cortical connections of lateral and medial portions of area 5 are distinct. Based on these data, 5L is closely associated with areas in anterior parietal cortex that process both cutaneous and proprioceptive inputs from the hands as well as motor cortex. Such inputs would be necessary for integration of forelimb posture, object properties and hand actions, a role suggested by functional studies of lateral area 5. In contrast, medial BA5 (MIP/PRR), proposed to code the intention to reach, has connections with regions of the brain associated with motor control, executive function and decision making. Further, the lack of connectivity from primary motor and anterior parietal cortex is consistent with medial BA5's putative higher-level involvement in motor control.

Disclosures: D.F. Cooke: None. **L. Krubitzer:** None. **J. Padberg:** None. **C.M. Cerkevich:** None. **J.H. Kaas:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.10/SS6

Topic: D.03. Multisensory

Support: LABEX CORTEX (ANR-11-LABX-0042) of Université de Lyon, within the program“Investissements d'Avenir” (ANR-11-IDEX-0007) operated by the French National Research Agency (ANR)

ANR-11-BSV4-501

Title: A quantitative analysis of the topology of subcortical projections to the macaque cortex

Authors: *A. R. RIBEIRO GOMES, C. LAMY, P. MISERY, C. DEHAY, K. KNOBLAUCH, H. KENNEDY;
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Abstract: Cortical areas can communicate directly, via cortico-cortical connections, and indirectly, via cortical subcortical cortical loops (CSCL). CSCL exist when the afferents to cortical area X in a subcortical structure are located in close proximity to the afferents to cortical area Y. The aim of the present study is two-fold: Firstly, to identify subcortical structures that participate in CSCL. The distance separating areas X and Y are expected to influence the existence of CSCL (Shipp et al., 2003). We have therefore examined CSCL with respect to two pairs of areas, a widely separated pair (areas 7m and 10) and a near by separated pair (areas V1 and V4). Secondly, to identify the range of structures projecting to each area and to quantify the projections of each. Two high sensitive and distinguishable retrograde tracers (FsB and DY) were injected in the target areas. High frequency plots of back-labeled neurons in subcortical structures were completed. The weight of the projection was expressed as the fraction of labeled neurons (FLNsc) in individual structures with respect to the total number of subcortical labeled neurons.
The subcortical input to areas V1, V4, 10, and 7m ranged between 3% and 8% of the total extrinsic input to these areas, the remaining 92% to 97% originated from the cortex. The two populations of neurons projecting to areas 7m and 10 were intermingled in the claustrum, intralaminar thalamic nuclei, mediodorsal thalamic nucleus, amygdala and basal forebrain. The two populations of back-labeled neurons following injections in area V1 and V4 were

intermingled in the claustrum, the pulvinar and the amygdala.
The strongest projection to both pairs of areas arises from the claustrum, followed by thalamus. Only in the case of V1 and V4 did a higher order thalamic nucleus form a strong CSCL. Given the high FLNsc value of the projection of the claustrum to all injected areas its CSCL could play an important role in long-distance communication between cortical areas (Crick& Koch, 2005).

Disclosures: A.R. Ribeiro Gomes: None. **C. Lamy:** None. **P. Misery:** None. **C. Dehay:** None. **K. Knoblauch:** None. **H. Kennedy:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.11/SS7

Topic: D.03. Multisensory

Support: NIH Grant T32AG020418-09

NINDS Grant T32NS041234-10

NINDS Grant 5R01NS061963-03

Title: Local and long-range connections from cortex and thalamus target specific, complementary sub-cellular compartments of corticospinal neurons

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Abstract: Corticospinal neurons (CSP) exhibit intriguing morphology: they form a prominent apical tuft in layer 1, and their perisomatic dendrites sample about half the thickness of cortex. We first quantified the three-dimensional dendritic arbors of identified corticospinal neurons (CSP) in mouse motor cortex (N=24) and found that dendritic length density across cortical layers is well predicted by somatic depth. The perisomatic and tuft compartments are separated by a thick apical trunk and may serve as substrates for a variety of computations, including coincidence detection. We wondered to what degree different projections target specific subsets of the corticospinal arbor and used optogenetic techniques to address this question. Retrograde tracing from motor cortex identified candidate origins of long-range input to corticospinal neurons. Anterograde viral delivery of channelrhodopsin-2 (ChR2) into thalamus or secondary somatosensory cortex (S2) resulted in photoexcitable axons in motor cortex; in utero electroporation delivered ChR2 to the major local input pathway (layer 2/3). Laser-scanning

photostimulation during whole-cell recordings from identified CSP revealed the subcellular localization of synapses. We found that synapses from local, long-range cortical (S2), and thalamic origins overlap in the perisomatic region, and target specific, complementary sub-regions of the apical CSP dendritic arbors. These morphological and synaptic mapping results together suggest that synaptic projections preferentially target not only specific subcellular compartments, but also specific sub-laminar populations of a single neuron class.

Disclosures: B.A. Suter: None. **G.M.G. Shepherd:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.12/SS8

Topic: D.03. Multisensory

Support: NIH Grant NS061963

Title: Selective connectivity of layer 6 corticothalamic neurons with other classes of projection neurons in mouse motor cortex

Authors: *N. YAMAWAKI[1], I. R. WICKERSHAM[2], G. M. G. SHEPEHRD[1];
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Abstract: Motor cortex (MC) contains 3 major classes of projection neurons: pyramidal tract (PT) neurons, exemplified by corticospinal neurons; intratelencephalic (IT) neurons, exemplified by callosally projecting corticostriatal neurons; and, layer 6 corticothalamic (CT) neurons, distinguished by their axonal projections to motor thalamus (VA/VL) and the thalamic reticular nucleus. In mouse MC, we investigated the connectivity of CT neurons with CT, IT, and PT neurons using a combination of optogenetic and electrophysiological methods. We injected deletion-mutant rabies virus encoding channelrhodopsin 2 and a fluorescent protein (RV-ChR2, Kiritani et al., 2012, J. Neurosci.) into motor thalamus, contralateral striatum, or spinal cord, to label CT, IT, or PT neurons, respectively. Inert fluorescent retrograde tracers were also injected in these locations, allowing us to perform whole-cell recordings from identified projection neurons in brain slices while photostimulating the ChR2-expressing population. Preliminary results indicate a striking paucity of excitatory connections between (layer 6) CT and (layer 5B) PT neurons. In contrast, both CT and IT neurons in layer 6 received excitatory inputs from IT neurons. These results, together with previous evidence (Kiritani et al., 2012), indicate that IT neurons are upstream to both CT and PT neurons, but CT and PT neurons are mostly not directly interconnected.

Disclosures: N. Yamawaki: None. **I.R. Wickersham:** None. **G.M.G. Shepehrd:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.13/SS9

Topic: D.03. Multisensory

Title: The control of firing pattern of midbrain periaqueductal gray neurons *In vivo*

Authors: *H. SUBRAMANIAN;
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Abstract: The midbrain periaqueductal gray (PAG) functions as an important component of the emotional motor system, as an integrator of behavioral responses. The PAG can be called the 'survival nucleus' because these behavioral responses are essentially coping strategies to face environmental and emotional challenges. In this context, the PAG maintains critical circuitries that control micturition, cardiovascular system, pain and analgesia and, lordosis. Respiration and vocalization being an essential activity for survival and emotional expression is also represented in the PAG (Subramanian et al., 2008; Subramanian, 2013; Subramanian and Holstege, 2013). However it is not known what type of activity patterns the PAG neurons express in vivo. In this study PAG was mapped for extracellular neuronal activity within its various subregions in vivo. Drugs were applied by direct microiontophoresis to activate silent cells and test their drug sensitivity.

In sodium barbiturate-anesthetized, spontaneously breathing and gallamine-paralyzed rats, PAG cells were found to be either nonfiring or sporadically firing. These cells were located in the lateral and ventrolateral PAG. The nonfiring PAG cells could be activated by iontophoresis of the excitatory amino acid glutamate. Cells made to fire in this manner ceased activity when glutamate ejection was terminated. Spontaneoulsy active cells were very few, restricted to the dorsal PAG region and these cells recorded extracellularly typically fired in a slow, irregular pattern. These were either non-bursting cells with a near normal distribution, or burst-firing cells typically showing a bimodal distribution.

Overall the PAG was found to be predominantly quiescent in the resting state.

However activation of the vagus or other peripheral respiratory and non-respiratory afferent interventions caused immediate activation of PAG neurons. In such instances, PAG cells showed two distinct types of activity patterns; 1) single spike firing and burst firing.

The functional implications of PAG neuronal activity are thus discussed in terms of descending motor and autonomic control.

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Disclosure.DisclosureBlock:

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.14/SS10

Topic: D.03. Multisensory

Title: Stimulation of melanocortin 4 receptors in the brainstem inhibits GABAergic/somatostatin interneuron activity

Authors: A. LEWIN, S. VICINI, R. A. GILLIS, *N. SAHIBZADA;
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Abstract: Melanocortin 4 receptors (MC4-R) have been extensively documented as being important regulators of energy balance. Their distribution corresponds well with forebrain and hindbrain nuclei that have been shown to influence food intake and body weight. In the hindbrain, of particular importance are nuclei that comprise the dorsal vagal complex (DVC). Of these nuclei, the nucleus tractus solitarius (NTS) and the dorsal motor vagal nucleus (DMV) are also key regulators of gastric motility. Recently (Richardson, et al., 2013), we reported that stimulation of MC4-R in the DVC differentially affects gastric motility via a vagally mediated circuit. In particular, stimulation of MC4-Rs in the NTS inhibits gastric motility, whereas their activation in the DMV excites it. The inhibition of gastric motility by MC4-R stimulation correlates well with reduction in food intake reported for MC4-R ligands injected into the NTS. Since gastric motility is controlled to a large extent by a GABAergic projection from the NTS to the DMV (Herman, et al., 2009) we wondered if our MC4-R stimulation was directly engaging this circuit or inhibiting a local inhibitory circuit. To test this, we performed cell-attached and whole-cell recordings from red fluorescent protein (RFP) labeled somatostatin (SOM) NTS

interneurons in brainstem slices of transgenic mice. In all neurons studied, bath application of α-MSH suppressed the neural activity of SOM neurons in the medial NTS. To further determine their role in the NTS-DMV axis, electrophysiological recording were made from DiI labeled gastric projecting DMV neurons in transgenic mice expressing channelrhodopsin-2-RFP in SOM interneurons. Stimulation of SOM interneurons in the NTS with light increased the firing frequency of the DMV neurons. This was in contrast to local stimulation of SOM interneurons in the DMV, which inhibited their firing. These preliminary findings suggest that α-MSH elicits its effect on gastric motility in the NTS by inhibiting GABAergic SOM interneurons. Furthermore, they suggest that these interneurons modulate the activity of premotor neurons of the DMV by either disengaging inhibitory output from the NTS or increasing local inhibition of the DMV.

Disclosures: A. Lewin: None. **S. Vicini:** None. **R.A. Gillis:** None. **N. Sahibzada:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.15/SS11

Topic: D.03. Multisensory

Support: MOST 2011CBA00400

NNSFC 31125015

NNSFC 31021063

Title: Oxytocin mediates early experience-dependent crossmodal plasticity of excitatory synaptic transmission in the sensory cortices

Authors: J.-J. ZHENG[1], S.-J. LI[1], X.-D. ZHANG[1], W.-Y. MIAO[1], *X. YU[2];
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Abstract: During the early postnatal period, neural activity, both in the form of spontaneous electrical activity and natural sensory stimulation, is critical to the formation of functional neural circuits. In addition to inducing changes within the target sensory cortex, sensory experience can also crossmodally affect other brain regions. Here, we report a novel form of plasticity in neonatal mice, where early sensory experience crossmodally regulates development of all sensory cortices via oxytocin signaling. Specifically, unimodal sensory deprivation from birth through whisker-deprivation or dark-rearing reduced excitatory synaptic transmission of layer 2/3 pyramidal neurons both in the correspondent sensory cortex, and crossmodally in other

sensory cortices. On the other hand, increasing natural sensory experience through environment enrichment significantly increased excitatory synaptic transmission in both barrel and visual cortices. Molecularly, cortical level of the neuropeptide oxytocin was bidirectionally regulated by sensory experience. Importantly, in vivo application of oxytocin elevated excitatory synaptic transmission in multiple sensory cortices, and significantly rescued the effects of sensory deprivation through oxytocin receptor mediated signaling. Together, these results further iterate the importance of multimodal sensory experience to early cortical development. We also identified a novel function for oxytocin signaling in promoting crossmodal, experience-dependent cortical development. The link between sensory experience and oxytocin signaling may be particularly relevant to autism spectrum disorders, where hyper- or hyposensitivity to sensory inputs is prevalent and oxytocin is a hotly debated potential therapy.

Disclosures: J. Zheng: None. **X. Yu:** None. **S. Li:** None. **X. Zhang:** None. **W. Miao:** None.

Poster

551. **Multisensory: Neural Circuitry and Connections**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 551.16/SS12

Topic: D.03. Multisensory

Support: DGAPA/UNAM IN200413

CONACYT 406644

Title: Ultrasonic vocalization in perinatally underfed pups, and massage stimulation effects

Authors: D. LOPEZ-JIMENEZ, M. REGALADO, *M. A. SALAS;
Developmental Neurobio. and Neurophysiol., UNAM, Queretaro, Mexico

Abstract: Undernutrition produces brain morphological alterations in some areas including the caudal nucleus ambiguus (Ambc). The Ambc innervates the intrinsic laryngeal muscles, which are involved in ultrasonic vocalizations (UVs) production. Early in life, the plastic changes and auditory experience can modulate the recognition of the frequency sound specificity by the auditory thalamocortical pathway. The integration of this information, let to reproduce a sound as UVs. Somatosensory pathway is closely related to the auditory pathway in the context that the Ambc hypoplasia caused by perinatal undernutrition (PU) can be partially reduced by massage stimulation (MS). The current study analyzed the UVs at 15 PN days in Control (CG), Underfeed (UG), Control stimulated (CSG) and Underfeed stimulated (USG) groups. MS was applied 10-min daily (days 4-15 of age). The UVs were obtained by an ultrasonic microphone and recorded

as an audible signal using a bat sound Software Spectrum Analyzer 3.0. Descending calls (n=10) were analyzed by spectrum power decomposition considered as frequency and intensity. The results, indicated that dominant frequency and power intensity scores were reduced ($p<0.05$) in the UG. The aim of this study was to demonstrate that MS could induce plastic changes to prevent the damage caused by PU in UVs.

Disclosures: D. Lopez-Jimenez: None. **M. Regalado:** None. **M.A. Salas:** None.**Poster**

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.01/TT1

Topic: D.04. Vision

Support: NIMH IRP

Title: Single unit activity in the monkey hippocampus related to short-term memory

Authors: *J. H. WITTIG, Jr., B. J. RICHMOND;
NIMH, BETHESDA, MD

Abstract: Single-unit activity was recorded from the hippocampus of one rhesus monkey as it performed a short-term memory task. Each trial started when the monkey grabbed a touch-sensitive bar. The task was to view a sequence of 2, 4 or 6 images on a computer monitor and report, by releasing the touch-sensitive bar either early or late, whether or not the last image of the sequence matched any of the preceding images. Each image was presented for 750 ms, with a 500 ms delay between successive images. Images were familiar and were presented repetitively throughout the testing session. In half of the trials the last image matched an image from the current sequence (early release rewarded), and in the other half of the trials it did not (late release rewarded). Behavioral performance was above 80% correct for each sequence length. We recorded the activity of 65 single units using up to 4 acute electrodes per daily testing session (25 total sessions). Approximately half of the recorded units (37 of 65) exhibited a significant change in spike rate during the last image that depended on whether or not the last image matched an image from the current sequence. For these units, an ANOVA showed that 6-45% of the trial-to-trial variance in spike rate during the last image was accounted for by match versus non-match status. We hypothesized that the observed change in spike rate reflected either a neural correlate of short-term memory of the last image (i.e., match versus non-match), or anticipation of the upcoming motor response (i.e., early versus late release). To control for the possibility of a motor-related signal, for 22 of the 65 units, we also recorded activity during a task that had the same motor requirements (early versus late release) but no short-term memory requirements

(response based on the identity of the last image, not whether it matched a preceding image). Approximately half of the units recorded during both tasks (12 of 22) had different spike rates during the last image when the task differed but the upcoming motor response was the same. We have identified three categories of single-unit activity in the monkey hippocampus immediately preceding the behavioral response to a remembered image: non-task, motor, and short-term memory related.

Disclosures: J.H. Wittig: None. **B.J. Richmond:** None.

Poster

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.02/TT2

Topic: D.04. Vision

Support: NIMH IRP

Title: Differential effects of TE and rhinal cortical lesions on serial recognition in rhesus monkeys

Authors: *M. A. ELDRIDGE, E. C. MASSEAU, R. C. SAUNDERS, B. J. RICHMOND; Lab. of Neuropsychology, NIMH, Bethesda, MD

Abstract: There is considerable evidence suggesting that area TE and the rhinal cortex of the inferior temporal cortex play an important role in the recognition of visual stimuli. Pharmacological, cooling and ablation studies have established a critical role for perirhinal cortex in delayed matching and non-matching to sample (DMS and DNMS) tasks. Neurophysiological recordings have found neural correlates for recency and familiarity memory within area TE, perirhinal cortex and entorhinal cortex.
We tested three TE-lesioned monkeys, three rhinal-lesioned monkeys (perirhinal + entorhinal) and three controls on a serial recognition task that did not require simultaneous comparisons between stimuli. In each trial the monkey had to indicate whether the stimulus displayed was presented for a first or second time within the session by releasing a lever in one of two intervals: either immediately after the stimulus appeared (first interval) or later when a small central target changed from red to green (second interval). Stimuli were repeated once within the session, with the interval being 0-128 stimuli. The stimulus set consisted of 6000 images of animals in natural scenes. No stimulus was reused within a 30-day period.
Performance was analyzed using signal detection theory. Control monkeys reliably differentiated between the first and second presentations of a stimulus at all but the longest intervals, 64 and

128 intervening stimuli. The ability of TE-lesioned monkeys to distinguish first and second presentations fell from a low level (considerably worse than controls) at short intervals (0 - 8 intervening stimuli) to chance levels beyond that. The performance of the Rh-lesioned monkeys was indistinguishable from that of controls, suggesting there are some types of recognition memory in which rhinal cortex may not play a role.

Disclosures: M.A. Eldridge: None. **E.C. Masseau:** None. **R.C. Saunders:** None. **B.J. Richmond:** None.

Poster

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.03/TT3

Topic: D.04. Vision

Support: NIMH IRP

Title: Modelling visual categorisation experimental observations from monkeys with a reinforcement learning-based spiking neural network

Authors: *S. CHANDRA[1], M. A. G. ELDRIDGE[1], F. P. HARTMANN[2], J. P. NADAL[2], B. J. RICHMOND[1];
[1]49 Convent Drive,, Natl. Inst. of Health, NIMH-LN,, Bethesda, MD; [2]Lab. de Physique Statistique, École Normale Supérieure, Paris, France

Abstract: Visual perceptual categorization is the process through which we assign objects into categories based on some similarity in their appearance. We have shown that monkeys easily learn to discriminate between cats and dogs in a small sample set, and to generalize this ability to never-before-seen exemplars. The perceptual difficulty of the discrimination was increased by 'morphing' together pairs of cats and dogs. This produced a set of stimuli with varying degrees of feature ambiguity (with cat:dog ratios of 100:0, 90:10, 80:20 ..... 0:100). The likelihood of a monkey assigning a cue to a particular category varies sigmoidally with the morph level of the cue. There is a small bias towards one category, presumably because rewards are asymmetrical, that is, only one category is rewarded (Eldridge, et.al., SfN 2012).
Here we present a reinforcement learning-based spiking neuron model reproducing the experimental observations. The model is a neural network with only input and output layers. The input layer neurons all have tuning curves of the same shape (eg. sigmoids, triangles, rectangles, etc.), each neuron with its own parameter values. Each input neuron represents one stimulus on a continuum of stimuli, i.e., the centers of the turning curves are ordered. The spikes from the

neurons of the input layer are summed with adjustable weights by the lone output layer neuron that uses a sigmoidal function to map the summed input into the probability of making the choice leading to reward. The network learns the adjustable weights through reinforcement learning with temporal discounting (in simulations we take temporal discounting over the last 5 trials). The predicted behavioral choices match the experimental findings in producing a sigmoidal performance curve, including the bias toward the rewarded category seen in the behavioral data. We study the model analytically and prove a universality property such that the predicted behavioral results are independent of the shape of the tuning curves of the neurons. Thus, it appears the form of the tuning curves for neurons for this simple type of reinforcement driven categorization need not play a critical role in learning simple perceptual categorization.

Disclosures: S. Chandra: None. **M.A.G. Eldridge:** None. **F.P. Hartmann:** None. **J.P. Nadal:** None. **B.J. Richmond:** None.

Poster

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.04/TT4

Topic: D.04. Vision

Support: CIHR

NSERC

CRC

Title: Anti-correlated spike rates associated with working memory activity in macaque dorsolateral prefrontal cortex

Authors: *M. LEAVITT[1], F. PIEPER[3], A. SACHS[4], J. MARTINEZ-TRUJILLO[2]; [2]Physiol., [1]McGill Univ., Montreal, QC, Canada; [3]Neurophysiol., Univ. of Hamburg-Eppendorf, Hamburg, Germany; [4]Dept. of surgery, Div. of neurosurgery, The Ottawa Hospital, Ottawa Hosp. Res. Institute, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Neurons in the primate dorsolateral prefrontal cortex (dlPFC) are known to exhibit selective activity during the delay period of spatial working memory (SWM) tasks. It has been hypothesized that functional interactions between these units may be involved in SWM maintenance, but whether and how these units interact with each other remains poorly understood. In order to investigate this issue, we recorded responses of multiple single units in

dlPFC area 8a of two macaca fascicularis using microelectrode arrays. The task consisted of fixation on a central spot for 494-800ms, then presentation of a circular sine wave grating at one of 16 randomly selected locations for 507ms. The offset of the grating was followed by a delay period that could last between 494-1500ms and ended with the offset of the central fixation point, cuing the animals to make a saccade to the remembered stimulus location. We recorded the activity of neurons in blocks of 32 channels and sorted spikes using Plexon software (Plexon Inc, TX). We isolated responses of 191 single units for a total of 1170 neuronal pairs. Neurons were classified as being selective (one-way ANOVA, $p<.05$) for the spatial location of the stimulus during the stimulus presentation period (visually-selective, n = 106, or 56%) and the delay period (memory-selective, n = 125, or 66%). We then computed spike-rate correlations and quantified the proportion of significant positive and negative spike rate correlations as well as the proportion predicted by chance (shuffled correlations) in each group for each task epoch. Correlations between stimulus-selective neurons and correlations between memory-selective neurons were greater than zero during the fixation and memory periods ($p < .05$, Sign test, Bonferonni-corrected), but not the stimulus period ($p > .05$, Sign test, Bonferonni-corrected). The proportion of significant positive correlations was also greater during the memory period than during the stimulus period ($p > .05$, chi-square test, Bonferonni-corrected) in both stimulus-selective neurons and memory-selective neurons. Importantly, the proportion of significant negative correlations was greater during the memory period than during the stimulus period ($p > .05$, chi-square test, Bonferonni-corrected) only between memory-selective neuronss. Our results suggest that interactions between neurons in the dlPFC increase during the maintenance of SWM, and these interactions vary based on neurons' selectivity.

Disclosures: M. Leavitt: None. **J. Martinez-Trujillo:** None. **F. Pieper:** None. **A. Sachs:** None.

Poster

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.05/TT5

Topic: D.04. Vision

Support: JSPS KAKENHI Grant Number 25830001

JSPS KAKENHI Grant Number 12J04982

JSPS KAKENHI Grant Number 23700489

Uehara Memorial Foundation

JSPS KAKENHI Grant Number 25870142

JSPS KAKENHI Grant Number 24220008

JST CREST

Title: MRI-assisted single-unit recording revealed precise neuronal map in the perirhinal cortices of macaque monkeys performing a visual pair-association task

Authors: *K. W. KOYANO[1], M. TAKEDA[1], T. MATSUI[1,2], Y. OHASHI[1], T. HIRABAYASHI[1], K. KAKIZAWA[1], T. WATANABE[1], Y. MIYASHITA[1,2];
[1]Dept. of Physiol., The Univ. of Tokyo Sch. of Med., Tokyo, Japan; [2]Dept. of Physics, The Univ. of Tokyo Sch. of Sci., Tokyo, Japan

Abstract: The perirhinal cortex is a part of the medial temporal lobe system and is known to be important for visual stimulus-stimulus associative memory. Although previous single-unit studies in this area have revealed activity patterns for each neuron, spatial profiles for these neurons, especially along the depth direction within the cortex, were difficult to determine because of technical problems with localizing recorded neural activities *in vivo* with fine resolution at a sub-millimeter scale. Here, we recorded single unit activities from the perirhinal cortices of two macaque monkeys performing a pair-association memory task, and precisely mapped the position of all the recorded activities at a resolution of 150 μm with the assistance of high-resolution structural magnetic resonance imaging (MRI) (Matsui et al. 2007, Koyano et al. 2011). In each recording session, we semi-chronically implanted a tungsten microelectrode at a target region and performed single-unit recording in an awake state and MRI scanning under anesthesia (a fast spin-echo sequence with a 4.7-T Bruker MRI scanner, TE/TR = 60/3000 ms) alternately on successive days. The recording track in each session was reconstructed from the microelectrode tip positions on the MRI images similarly to the way that conventional acute-experiment studies reconstruct from electrolytic lesions on histological sections. The reconstructed neuronal map (n = 1145 neurons) revealed a cluster of neurons selective for visual cue stimuli (one-way analysis of variance, $p<0.01$) within area 36 of the perirhinal cortex, shaped like a cylindrical column. Within the cluster, the stimulus-selective neurons located in the deeper region frequently showed strong stimulus selectivity during the delay period following a cue-stimulus presentation, while those located in more superficial region showed the delay selectivity only occasionally. These results indicate that neurons in the perirhinal cortex were not randomly distributed but were spatially organized according to their response properties both in terms of their cylindrical clustering within the perirhinal cortices as well as their functional dissociation across the cortical depth within the cluster.

Disclosures: K.W. Koyano: None. **M. Takeda:** None. **T. Matsui:** None. **Y. Ohashi:** None. **T. Hirabayashi:** None. **K. Kakizawa:** None. **T. Watanabe:** None. **Y. Miyashita:** None.

Poster

552. Visual Cognition: Memory

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.06/TT6

Topic: D.04. Vision

Support: Japan Society for the Promotion of Sicence (JSPS) through its FIRST program

Title: Dissociation between behavioral flexibility and persistent memory of cue-reward contingency represented by the activities of perirhinal (PRh) neurons in macaque monkeys

Authors: *M. K. ERADATH[1,2], T. MOGAMI[1], K. TANAKA[1];
[1]Lab. for Cognitive Brain Mapping, Brain Sci. Institute, RIKEN, Saitama, Japan; [2]Joint Frontier Studies, Grad. Sch. of Sci. and Engineering, Saitama Univ., Saitama, Japan

Abstract: To clarify the nature of reward-related activities in PRh cortex, we recorded responses of PRh cells under a task in which the cue-reward contingency changed depending on the contextual time frame. Visual cue presentation and reward delivery were repeated twice in each trial, in a sequence of cue, reward (or no reward), cue, reward (or no reward) with temporal delays at each interval. The monkeys had to keep pressing a bar and fixating the gaze until the timing of second reward delivery was over. There was a fixed cue-reward contingency in the first part of trial (half of 24 cues predicted reward, and the remaining cues predicted no reward), whereas reward was randomly provided in the second part. The gaze-fixation break frequency and water-tube-sucking strength showed that the monkeys anticipated the reward condition in the first part, but not in the second part. Responses of PRh cells to reward-contingent cues were different from those to no-reward-contingent cues in the first part, but not in the second part. Thus, it has been shown that activities of PRh cells integrate the cue-reward contingency with the contextual time frame. After several months of recordings, we shifted the cue-reward contingency from the first to second part of trial: the reward delivery was now random in the first part but contingent on cues in the second part. The monkeys' behavior followed the change within a few days, whereas many PRh cells continued to exhibit differential responses in the first part for about a month. Reward-anticipatory activities eventually moved to the second part after this period. These results suggest that the reward-anticipatory PRh activities are not a simple reflection of monkeys' anticipation or motivation, but are more persistently dependent on the past long-term experiences. Finally, we introduced the contextual change within each daily session. The cue-reward contingency existed only in the first part of trial for the first 400 trials, and then moved to the second part for the remaining trials of the day. The monkeys' behavior followed the contextual change within 100 trials. Many PRh cells showed reward-anticipatory activities in the contingent part only before or after the contextual change, although some others

followed the contextual change. The quick switch of monkeys' behavior might occur by alternatively turning on two groups of PRh cells that memorized cue-reward contingency in different parts of trial.

Disclosures: M.K. Eradath: None. **T. Mogami:** None. **K. Tanaka:** None.

Poster

552. Visual Cognition: Memory

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.07/TT7

Topic: D.04. Vision

Support: NSF DGE-1122492

NIH R01 EY014193

Title: Timescale limitations of perceptual expectation on fMRI repetition suppression

Authors: *E. J. WARD, M. M. CHUN;
Yale Univ., New Haven, CT

Abstract: Predictive coding theories propose that the brain actively anticipates forthcoming stimuli and events (e.g. Friston, 2005), reducing processing demands when sensory evidence matches a predictive template and generating an error signal to increase processing when input deviates from the template. One fMRI measure of processing demands is repetition suppression, in which the fMRI signal is lower for repeated items. Thus, when a stimulus is predictable (such as when it is more likely to repeat), predictive coding accounts posit larger repetition suppression (greater amplitude difference between first and subsequent presentations) -- prior studies confirmed that increased probability and expectation of a repetition resulted in greater repetition suppression for images repeated in quick succession (Summerfield et al., 2008). Repetition suppression can occur across short and long repetition intervals, even with many intervening stimuli, but the mechanisms that drive the effect may differ for different timescales (Epstein et al., 2008). Therefore, in this study, we investigated whether perceptual expectancy, manipulated by varying the probability of stimulus repetition, can modulate repetition suppression at long intervals and with intervening items. We scanned 22 participants while they viewed sequences of scenes, many of which repeated after 7 seconds (no intervening scene) or 17 seconds (one intervening scene) at either 25% or 75% probability. We measured repetition suppression between the first and second scene presentations. In the parahippocampal place area (PPA), repetition probability (perceptual expectation) did not affect the magnitude of repetition

suppression, indicating that perceptual expectation effects at short lags may not extend to long intervals. These results have implications for models of short- and long-interval repetition suppression and suggest timescale limitations for theories of predictive neural coding.

Disclosures: E.J. Ward: None. **M.M. Chun:** None.

Poster

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.08/TT8

Topic: D.04. Vision

Support: EUREKA

NIH

Title: Reactivation of visual activity in human electrocorticography

Authors: *B. J. HANSEN[1,2], M. I. CHELARU[2], N. TANDON[3], C. R. CONNER[3], S. SZUKALSKI[2], J. D. SLATER[2,4], G. P. KALAMANGALAM[4], V. DRAGOI[2];
[1]Systems Neurobio. Lab., The Salk Inst., La Jolla, CA; [2]Neurobio. and Anat., [3]Neurosurg., [4]Neurol., UT Med. Sch., Houston, TX

Abstract: One of the fundamental questions in neuroscience is to understand how encoding of sensory inputs is maintained across neuronal networks in cerebral cortex to influence sensory processing and behavior. We examined the brain's remarkable ability to spontaneously evoke previous visual experience in the absence of sensory input. Published studies using single-cell electrophysiology in cat visual cortex and monkey visual area V4 have shown that ongoing activity resembles previously evoked responses to natural movies or image sequences (Yao et al., Nature Neuro, 2007; Eagleman & Dragoi, PNAS, 2012). Surprisingly, given reactivations fundamental role in sensory processing whether and how reactivation occurs in human cortex is unknown. We decided to explore reactivation in the context of human electrocorticography (ECoG) recordings. We measured neuronal activity as changes in the local field potentials in four human patients and assessed reactivation (number of electrodes = 444) across multiple frequency bands in response to dynamic visual stimulation. The experiment consisted of multiple trials in which human subjects were exposed to either short cartoon movies or blank screens. Each session began and ended with 25 blank fixation trials (pre and post), while the visual sequence consisted of 50 interleaved movie and blank trials. On all trials, subjects were required to maintain fixation in the center of a computer screen while performing a letter identification

task. To evaluate cortical reactivation we assessed the degree of similarity between the ECoG signals filtered across frequency bands. Specifically, we computed the distance between the Hilbert amplitude sequences during pre and movie, D(pre, movie), and between the HA sequences during movie and blank, D(movie, blank). If D(movie, blank) was significantly higher than D(pre, movie) across trials, the electrode was considered to exhibit cortical reactivation. We observed that, irrespective of cortical location, the highest percentage of reactivation electrodes (~ 60%) was found in the gamma-band (32-128 Hz). Is reactivation localized to a specific cortical region? We observed the highest percentage of reactivation electrodes (~ 44%) in the frontal (N = 35) and temporal lobes (N = 29). Numerous control experiments assessed the degree of reactivation during the inter-trial-interval, in response to different movie/blank conditions and the capacity of signal in during the post. Our results indicate a surprising degree of plasticity in human cortical networks in the absence of visual stimulation that may constitute the neural basis of learning by stimulus exposure in the absence of attention.

Disclosures: B.J. Hansen: None. **N. Tandon:** None. **C.R. Conner:** None. **M.I. Chelaru:** None. **S. Szukalski:** None. **J.D. Slater:** None. **V. Dragoi:** None. **G.P. Kalamangalam:** None.

Poster

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.09/TT9

Topic: D.04. Vision

Support: NIH(NEI) Grant R01EY020958

Title: A resource view on visual short-term memory for multi-feature objects

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Abstract: Studies of visual short-term memory (VSTM) for multi-feature objects have typically asked three questions: 1) Do observers remember integrated objects or individual features? 2) Can observers remember the same number of features regardless of how many objects they belong to? 3) Do salient irrelevant features affect performance? These questions are often studied in the framework that VSTM has a discrete, limited capacity. In an alternative view, VSTM resource is a continuous quantity; the more resource a feature receives, the higher its precision. Within this new framework, we reformulate the above questions as: 1) Is resource shared between features? 2) Are the resources for different features independently allocable to objects?

3) Can salient irrelevant features be ignored during decision-making?
We used the same change localization paradigm to answer all three questions. In Experiment 1, human subjects briefly viewed four ellipses with random orientations and colors. After a 1-second delay, a second display containing four ellipses appeared, exactly one of which had changed. In the one-relevant-feature condition, the change occurred always in the same feature - either orientation or color. In the two-feature condition, the change occurred randomly in either feature. Observers reported the location of the change.
We found that psychometric curves were identical between both conditions, suggesting that VSTM resource is not shared by features. To rule out that resource is shared by features but the irrelevant feature automatically takes up resource, we compared the one-relevant-feature condition with a truly-one-feature condition that used colored discs or grey ellipses. Performance was not affected by the presence of the irrelevant feature.
To address the second question, in Expt. 2, we compared the two-feature condition from Expt. 1 to a condition in which the features of each item are spread out over two objects (producing 8 single-feature objects) and to the two-feature condition with set size 8. We found that performance in the 'spread-out' condition lied in between performance in both two-feature conditions, suggesting that some but not all feature resources are allocated together.
Finally, we compared the one-relevant-feature condition from Expt. 1 to a condition in which all objects change in the irrelevant feature and only one changes in the relevant feature. Performance was identical between both conditions, suggesting that observers can ignore changes in the irrelevant feature.
We conclude that there exist independent pools of VSTM resource for orientation and color, but they are distributed over objects in a partially dependent manner.

Disclosures: H. Shin: None. **R. van den Berg:** None. **W. Ma:** None.

Poster

552. **Visual Cognition: Memory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 552.10/TT10

Topic: D.04. Vision

Support: ARC Grant DP1093279

ARC grant CE110001021

Title: Contrasting left and right perirhinal cortex recruitment during visual processing of verbal and non-verbal stimuli

Authors: *M. A. DALTON, M. HORNBERGER, J. R. HODGES, O. PIGUET;
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Abstract: Background The medial temporal lobes (MTL) contain structures that are crucial for memory processing including the hippocampus, parahippocampal gyrus and perirhinal cortex. Recent evidence suggests that the perirhinal cortex sits at the apex of the ventral visual processing stream and is involved in binding higher order representations of complex visual stimuli. As such, in addition to mnemonic processing, this region is implicated in perceptual processing. In addition, functional neuroimaging and clinical lesion studies indicate that information in the MTL may be lateralised depending on the type of material being processed, with left and right MTL preferentially engaged in processing verbal and non-verbal stimuli respectively. While functional neuroimaging studies have provided support for each of these positions, no study has directly contrasted MTL contributions to visual processing of semantic and non-semantic stimuli in the same group of subjects. The aim of this study was to determine the extent to which the perirhinal cortex is involved in visual processing of verbal and non-verbal stimuli. **Methods** Twenty right-handed adults (6 females; mean age = 26 years) participated in an fMRI experiment comprising two memory tasks: (i) a recognition memory task using compound words, (ii) a recognition memory tasks using complex symbols. The neural correlates of visual processing for each stimulus type were analysed using fMRI data from the encoding phase of each task. The fsl-feat software package was used to analyse the data. **Results** We observed increased BOLD signal in the left perirhinal cortex during visual processing of compound words. In contrast, increased BOLD signal was observed in the perirhinal cortex bilaterally during visual processing of complex symbols with a greater cluster size and signal strength in the right perirhinal cortex. We found no evidence for recruitment of other MTL substructures in either condition. **Conclusions** The results of this study provide evidence that (i) the perirhinal cortex is recruited during visual processing of verbal and non-verbal stimuli and (ii) that there is asymmetrical recruitment of the left and right perirhinal cortex during perception of verbal and non-verbal stimuli respectively. These findings have important implications for theoretical models of MTL involvement in visual processing.

Disclosures: M.A. Dalton: None. **M. Hornberger:** None. **J.R. Hodges:** None. **O. Piguet:** None.**Poster**

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.01/TT11

Topic: D.04. Vision

Support: NEI EY17934

Title: Biophysical mechanism of the omitted stimulus response

Authors: *N. DESHMUKH[1], F. S. SOO[1], G. W. SCHWARTZ[3], M. J. BERRY, 2[2];
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Abstract: The retina constitutes the earliest stage of visual processing. Surprisingly, even without feedback from the brain, the retina is capable of detecting complex stimulus patterns. Retinal ganglion cells respond to interruptions of periodic flash sequences with an extra burst of action potentials. Over a broad range of flash train frequencies, the latency of this Omitted Stimulus Response (OSR) corresponds precisely to the time of the missing flash (Schwartz *et al., Nat Neurosci.* 2007). However, the biophysical mechanisms by which the retina generates this extra response and entrains to the frequency of the flash sequence have not been determined. Several competing models have been proposed to explain the origin of the OSR. One class of models proposes a frequency-tunable resonant element in ON bipolar cells that continues to oscillate when the flash sequence is interrupted. Ganglion cells combine this additional oscillatory input from the ON pathway with the OFF pathway to generate the OSR (Gao *et al., Network* 2009). An alternative hypothesis is that appropriately timed OSRs in the input currents of ganglion cells are simply due to temporal integration of several flashes and can be explained by a simple linear-nonlinear model with a spike threshold. (Werner *et al., J. Neurophysiol.* 2008). In order to distinguish between these and other possibilities, we characterized the responses of individual ganglion cells and bipolar cells to single flashes and interruptions of flash trains using whole-cell patch clamp recording.
Voltage-clamped bipolar cells show strong adaptive gain control during periodic flash sequences and can generate an extra synaptic current in response to both single flashes and the interruption of a flash train. These nonlinearities in the input current are not consistent with either model of the OSR. Additionally, the timing of this extra response does not depend on stimulus period. In contrast, voltage-clamped retinal ganglion cells exhibit strong OSRs in their excitatory input currents. These data suggest that a period-dependent transformation of the extra response occurs at the bipolar to ganglion cell transfer.

Disclosures: N. Deshmukh: None. **F.S. Soo:** None. **G.W. Schwartz:** None. **M.J. Berry:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.02/TT12

Topic: D.04. Vision

Support: NS003039

Title: The role of bipolar cells in direction selectivity circuitry examined by a novel fluorescent labeling technique

Authors: *A. POLEG-POLSKY, J. S. DIAMOND;
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Abstract: Retinal on-off Direction Selective Ganglion Cells (DSGCs) respond to images moving in a specific direction across the retina. This computation is thought to rely on directionally tuned input from Startburst Amacrine Cells (SACs) and Bipolar cells. In a previous study we postulated that tuning of bipolar cells' input may be an artifact of inadequate space clamp when recording DSGC activity with a somatic patch electrode.
To probe directly whether bipolar cells are directionally selective, we developed a novel technique of loading bipolar cells with fluorescent indicators by a mechanical tissue manipulation. This new method produces very bright and sparse labeling of individual bipolar cells while preserving light responses. By filling the cells with OGB-1 and performing two-photon imaging from axon terminals of bipolar cells and dendrites of patched DSGCs we could simultaneously record pre- and post-synaptic calcium signals and EPSPs. Our initial findings are consistent with directionally unselective bipolar signals.
To examine how synaptic inputs from multiple sources are integrated in a DSGC we performed a multi-compartmental computer simulation of a DSGC. The simulation revealed that directionally unturned glutamatergic inputs from bipolar cells can produce tuned NMDAR currents due to postsynaptic interactions with inhibitory inputs from SACs. Our simulation suggests that NMDAR conductance can uniquely increase the gain of a DSGC without reducing the direction selectivity of the response.

Disclosures: A. Poleg-Polsky: None. **J.S. Diamond:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.03/UU1

Topic: D.04. Vision

Support: NEI grant EY017992

NSF DBI-1003198

Volkswagenstiftung

BWF CASI

Title: Model-based analysis of an electrically-coupled amacrine cell network in primate retina

Authors: *A. K. HEITMAN[1], M. GRESCHNER[2], G. D. FIELD[3], P. H. LI[1], D. AHN[1], A. SHER[4], A. M. LITKE[4], E. J. CHICHILNISKY[1];
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Abstract: Amacrine cells are the most diverse cell class in the retina, and many of the ~25 distinct types are poorly understood. Using dense multi-electrode recordings in primate retina, we recently discovered a novel polyaxonal amacrine cell (PAC) type via its electrical coupling to ON-parasol ganglion cells. To understand how this PAC type shapes visual signals transmitted to the brain, here we introduce model-based quantitative analysis of inhibitory interactions between PACs and ON-parasol cells during visual stimulation.
PACs were identified using the electrical image, i.e. the average voltage deflection across the multi-electrode array during a spike (Litke et al, 2004). The electrical image revealed the soma and axon of recorded cells. However, additional structure in the electrical image also revealed multiple outwardly radiating processes, likely corresponding to axons of PACs electrically coupled to ON-parasol cells (Dacey & Brace, 1992). Analysis of spontaneous activity revealed negative correlations between ON-parasol cells coupled to PACs and other ON-parasol cells near the PAC axons. Thus, PACs mediate lateral inhibition in the ON-parasol network.
To understand the functional impact of PAC inhibition, we developed model-based quantitative analysis of network interactions during visual stimulation. A generalized linear model (Pillow et al, 2008) was fitted to ON-parasol cell spike trains, capturing their dependence on the visual input and their association with simultaneously recorded spikes from PACs coupled to ON-parasols. During a white noise stimulus, negative correlations between distant ON-parasols were undetectable, presumably due to larger stimulus driven response fluctuations. However, clear inhibitory interactions lasting 2-3 ms were observed as feedback terms in model fits. To understand the effects of this inhibition during structured visual stimulation, responses to a central (excitatory) grating with a peripheral (suppressive) grating were recorded. Comparison to simulations suggested that each ON-parasol cell receives inhibitory input from tens of PACs. These results indicate that an electrically coupled network of PACs provides homotypic surround suppression to a major pathway in the primate visual system.
Support: NEI , Volkswagenstiftung, NSF, BWF CASI

Disclosures: A.K. Heitman: None. **M. Greschner:** None. **G.D. Field:** None. **P.H. Li:** None. **D. Ahn:** None. **A. Sher:** None. **A.M. Litke:** None. **E.J. Chichilnisky:** None.

Poster

553. Retinal Circuitry: Synaptic Interactions

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.04/UU2

Topic: D.04. Vision

Support: MEXT Grant SENRYAKU 1001034

Title: Morphology of tyrosine hydroxylase-immunoreactive amacrine cells in the retina of developing gerbil

Authors: H. IMADA[1], K. SAKAI[2], *E.-I. MIYACHI[1];
[1]Dept Physiol, Fujita Hlth. Univ. Sch. Med., Toyoake, Aichi, Japan; [2]Dept Anat, Fac Clin. Engin, Fujita Hlth. Univ. Sch. Hlth. Sci., Toyoake, Aichi, Japan

Abstract: The development of dopaminergic neurons in the mammalian retina has been studied by using immunocytochemical method with antibody against tyrosine hydroxylase (TH) . We examined the shape, localization and cell population change of the TH-immunoreactive (TH-IR) amacrine cells in the gerbil (*Meriones unguiculatus*) retina from postnatal day (P) 1 to adult. Gerbils were given a dose of Nembutal, and then perfused transcardially with 0.1M phosphate buffer (PB; pH 7.4) for wholemount preparations, with 0.1M phosphate buffer added to 4% buffered paraformaldehyde for sections. After removed eyeballs were frozen with a rapid jet of liquid carbon dioxide, they were cut with section of 16μm using a cryostat. The sections and wholemount preparations were incubated in rabbit anti-tyrosine hydroxylase for 24h at 4°C. They were examined by ABC (avidin-biotin-peroxidase complex) staining method. We have found two kinds of TH-IR amacrine cells (type A and type B). The two types were clearly different in the shape of somata and stratification of TH-IR dendrites in the inner plexiform layer (IPL) at P7. Type-A TH-IR cells had monostratified dendrites extending in the outermost layer of the IPL, while type-B cells had dendrites extending in the middle of IPL. At P7, type-A somata were observed in the inner part of the inner nuclear layer (INL), its dendrites extended into the outer part of the IPL. Type-B somata were located in the inner part of the newly formed INL. Its dendrite was observed to spread in the middle of the IPL. At P14, type-A somata had grown considerably. The stem dendrites branched from the thickly stained and larger pyriform soma, spreading horizontally only in the outermost of the IPL. Type-B somata had also grown considerably, the dendrites went through TH-IR fibers of the outer part of the IPL, and then spread in the middle of the IPL. At P28, type-A somata became spherical and significantly larger, and more thickly stained, located in the vicinity of outermost layer of the IPL. The dendrites of type B extended in the middle of the IPL, where the point-like layer was observed.

In adult type A, densely stained, round-shaped and large somata were located in the innermost part of the INL, and their dendrites with varicosities extended into the outermost part of the IPL. Adult type-B somata, on the other hand, were stained weakly, with their thin dendrites penetrating the outer part of the IPL. These results suggest that two kinds of dopaminergic amacrine cells have different developmental properties in the developing gerbil retina. Eye opening is an important period for the maturation of dopaminergic amacrine cells and for the maturation of the IPL.

Disclosures: H. Imada: None. **K. Sakai:** None. **E. Miyachi:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.05/UU3

Topic: D.04. Vision

Support: European Commission FP7 Grant RETICIRC HEALTH-F2-2009-223156

ALW-NWO

ZonMW-NWO

Title: Feedback from horizontal cells to cones: An unexpected synthesis of an ephaptic and a pH-mediated system

Authors: *R. VROMAN, L. KLAASSEN, M. HOWLETT, J. KLOOSTER, T. SJOERDSMA, M. KAMERMANS;
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Abstract: Horizontal cell (HC) to cone photoreceptor negative feedback generates the centre-surround organization of the bipolar cells. The mechanism behind this inhibitory communication has been a matter of debate in the last decades and is mainly centred on two hypotheses: an ephaptic and a proton-mediated mechanism. We show that negative feedback is mediated by an unexpected synthesis of both mechanisms. The ephaptic mechanism is extremely fast without a delay and does not add any significant filtering to the signal. The pH modulation mechanism is much slower, with a time constant of 189 ± 25 ms and depends on the release of ATP via Pannexin 1 (Panx1) channels located on the HC dendrite. Instead of acting on purinergic receptors, the ATP is extracellularly hydrolysed to AMP, phosphate groups and protons by the ecto-ATPase NTPDase1. The phosphate groups and protons form a pH buffer with a pKa of 7.2,

acidifying the synaptic cleft. This leads to an inhibition of the presynaptic calcium channels and consequently decreased glutamate release from the photoreceptor. Hyperpolarisation of the HCs closes the Panx1 channels leading to a decrease in pH buffer capacity resulting in an alkalinisation of the synaptic cleft and disinhibition of the calcium current. The result is an increase in glutamate release. Our findings not only resolve a longstanding controversy in retinal research, it also shows a fully novel form of synaptic modulation. Since Pannexin 1 and NTPDase1 are abundantly expressed throughout the central nervous system, it is likely that this mechanism is more widely spread than just in the first retinal synapse.

Disclosures: R. Vroman: None. **L. Klaassen:** None. **M. Howlett:** None. **J. Klooster:** None. **T. Sjoerdsma:** None. **M. Kamermans:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.06/UU4

Topic: D.04. Vision

Support: NIH Grant EY15573

UCLA Oppenheimer Seed Grant

Plum Foundation

Canadian Institutes of Health Research

National Science and Engineering Research Council

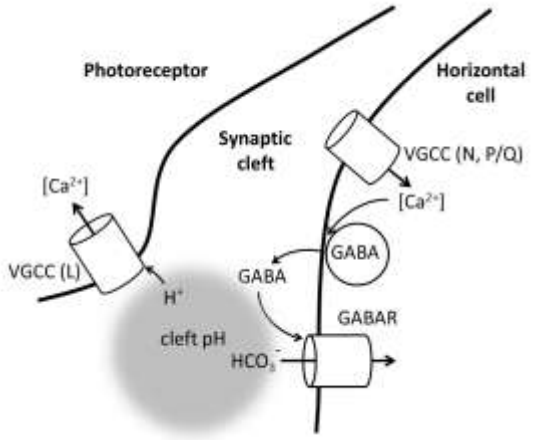
Veterans Administration Senior Career Scientist Award

Title: Horizontal cells inhibit photoreceptors via an unconventional GABA- and pH-sensitive mechanism in rat retina

Authors: X. LIU[1], A. A. HIRANO[1], X. SUN[1], N. C. BRECHA[1], *S. A. BARNES[2]; [1]UCLA, Los Angeles, CA; [2]Dalhousie Univ., Halifax, NS, Canada

Abstract: Horizontal cells send inhibitory feedback to photoreceptors, helping form centrer-surround antagonistic receptive fields in the retina, but the neurotransmitter and the mechanisms underlying this signalling are not known. The proteins responsible for conventional Ca^{2+}-dependent release of GABAergic synaptic vesicles are present in mammalian horizontal cells so

we investigated this mechanism as the means by which horizontal cells inhibit photoreceptors. Using Ca^{2+} imaging in rat retinal slices, we confirmed that horizontal cell depolarization with kainate inhibits and horizontal cell hyperpolarization with NBQX disinhibits the Ca^{2+} signals produced by activation of Ca channels in photoreceptors, actions that were blocked by HEPES. While 100 μM Co^{2+} reduced photoreceptor Ca^{2+} signals, it disinhibited them at 10 μM, an effect reminiscent of earlier studies where low [Co^{2+}] eliminated feedback. The low [Co^{2+}] disinhibition was pH-sensitive, being eliminated by HEPES. We localized L-, N-and P/Q-type Ca channels in rat horizontal cells, and showed that both the N-type Ca channel blocker ω-conotoxin GVIA and the P/Q-type Ca channel blocker ω-agatoxin IVA increased Ca^{2+} signals in photoreceptors in a pH sensitive manner. Pronounced actions of GABAergic agents on feedback signals to photoreceptors were observed, and are pH-sensitive, but are inconsistent with direct inhibition by GABA of photoreceptor [Ca^{2+}]. Patch-clamp studies revealed that GABA activates a conductance having high bicarbonate permeability in isolated horizontal cells, suggesting that the commonality of pH-sensitivity throughout these results could arise from a GABA autofeedback action in horizontal cells. This conductance increase could change cleft pH with concomitant inhibitory influences on photoreceptor Ca channels.



Disclosures: X. Liu: None. **A.A. Hirano:** None. **X. Sun:** None. **N.C. Brecha:** None. **S.A. Barnes:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.07/UU5

Topic: D.04. Vision

Support: 1R25GM097636

Title: Evaluating post synaptic densities and ribbon synapses in mouse models of glaucoma

Authors: *T. A. JENRETTE[1], C. BENOIST[2];
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Abstract: The Calkins lab works toward the overall goal of better understanding both why and how glaucoma causes vision loss. Glaucoma has been described as a disorder for which the major risk factor is elevated intraocular pressure (IOP) leading to damage of the optic nerve. This however, is a simple explanation for a complex problem. Current glaucoma treatments focus on lowering the IOP and not on preserving the cells in the retina and their function. Because treatment is not aimed at preserving or recovering the function of retinal cells, glaucoma eventually progresses and results in blindness. Additionally, lowering IOP fails to aid patients suffering from normal tension glaucoma, a form of glaucoma in which IOP is not elevated but progression of the disease is the same. A better understanding of the mechanisms underlying retinal degeneration in glaucoma would make preventative or regenerative treatment options possible. Previous research has shown that RGCs undergo significant and detrimental changes during the early stages of glaucoma, and that there is an inverse relationship between elevated IOP and the number of retinal ganglion cell (RGC) bodies, dendrites and synapses in the retina. In this experiment, we evaluated the number of RGC synapses in the retinas of DBA/2J and microbead injected C57 mice by performing immunohistochemistry with antibodies specific for the synaptic proteins post-synaptic density protein-95 (PSD-95) and RIBEYE. DBA/2J mice are a strain that is predisposed to two forms pigmentary glaucoma (IPD and ISA). Glaucoma occurs in these mice as a result of the crumbling of the iris pigment or iris stroma that blocks aqueous humor drainage and elevates IOP. In the C57 mouse model (MOM) glaucoma is induced via microbead injection into one eye to block outflow and increase IOP. The other eye receives a saline injection and thus serves as the control. In either model, the elevated pressure damages the retina and allows us to examine the effect of IOP on RGC synapses. Confocal images indicated a decrease in the number of PSD-95 and RIBEYE positive synapses in retinas with elevated IOP. These data suggests a correlation between IOP and the number of synapses present. Future studies will reveal more information about specific signaling events that lead to synaptic loss.

Disclosures: T.A. Jenrette: None. **C. Benoist:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.08/UU6

Topic: D.04. Vision

Title: The interplay between proton gradients and chloride flux in retinal amacrine cells

Authors: *V. S. KRISHNAN, E. GLEASON;
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Abstract: Previous research from our lab has shown that nitric oxide (NO) releases Cl^- from an internal store and that the release is dependent upon a transient acidification of the cytosol (Hoffpauir 2006; McMains and Gleason 2011). Our strongest candidates for internal Cl^- stores are endosomal compartments which contain 20-60 mM Cl^- and maintain a low pH via the V-type H^+ pump. Furthermore, coupled antiport of H^+ and Cl^- has been shown for ClC transporters which are known to reside on endosomal membranes. Here we test the hypothesis that the distribution of protons across internal membranes influences the ability of NO to release internal Cl^-. We have previously reported that the weak bases methylamine (MA, 10mM) and chloroquine (CQ, 100µM) reliably reduced the LysoSensor fluorescence signal. This effect is likely due to the buffering action of MA and CQ in acidic compartments such as endosomes where LysoSensor accumulates. To test the consequences of buffering on the NO-dependent release of internal Cl^-, we made whole cell voltage clamp recordings from individual chick amacrine cells in culture. Voltage ramps were delivered in the presence of GABA (20µM) to activate $GABA_A$ and the reversal potential ($GABA_{rev}$) of the current was measured under different conditions. The initial reversal potential of the GABA-gated current was more positive in MA and CQ (internal solution) than under control conditions (p=0.01 for both) suggesting that cytsolic Cl^- was elevated when endosomal pH was buffered. After NO, $GABA_{rev}$ moved negative under buffering conditions suggesting that cytosolic Cl^- was taken up into the store or expelled from the cell. To test whether the H^+ gradient across internal membranes was required for the NO-dependent release of internal Cl^-, we used the protonophore carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, 1µM) to collapse H^+ gradients and bafilomycin (1µM) to inhibit the V-type H^+ pump. In LysoSensor experiments, we consistently observed a reversible reduction in LysoSensor fluorescence with both reagents consistent with H^+ leaking from acidic compartments. In voltage clamp experiments, we held amacrine cells at -70mV under Cl–free internal and external conditions to isolate the Cl^- store. Pulses of GABA (20µM, 400msec) were applied to test whether cytosolic Cl^- was available to carry an inward current. Under control conditions, no GABA-gated currents were observed until NO was applied. In FCCP external solution, the NO-dependent release of internal Cl^- was not observed. These results suggest that the H^+ gradient across internal membranes is an important component in the machinery that controls the NO-dependent release of internal Cl^-.

Disclosures: V.S. Krishnan: None. **E. Gleason:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.09/UU7

Topic: D.04. Vision

Support: NIH Grant EY017428

Title: Circadian-induced AMPA Receptor plasticity in retinal ganglion cells

Authors: *M. D. PEDISICH, S. NAWY;
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Abstract: The retina is a dynamic tissue that can process a wide range of visual inputs. To do so, network and synaptic properties of retinal cells must be adaptive. Long term adaptations can take place in accordance to circadian rhythm and a number of processes in the retina oscillate with a day/night cycle. AMPA receptor (AMPAR) composition and membrane density are key to plasticity in many parts of the central nervous system, including in the cortex, hippocampus, and cerbellum, and may play a similar role in the retinal ganglion cell (RGC) membrane. All signals in the retina converge on RGCs, which are the sole output of the retina. Previous work has suggested that short-term changes in AMPAR subunit composition can adjust the sensitivity of RGCs to light. Furthermore, AMPARs have been shown to be able to switch from a cycling state to a non-cycling state depending on long-term activity. Here, our goal is to understand how AMPAR cycling in RGCs is induced and how it affects the function of RGCs in the retinal circuit. A change in AMPAR cycling states in the membrane of RGCs is circadian-driven and relies on rod/cone photoentrainment. Further, receptor cycling induces changes in the synaptic properties of the RGCs which change their sensitivity to light. RGCs during subjective night, expressed an smaller relative number of GluR2-containing AMPARs while RGCs during subjective day mediated AMPAR response almost entirely via GluR2-containing AMPARs. This phenomenon persisted in a circadian-driven fashion and affects the relative light sensitivity of the RGC.

Disclosures: M.D. Pedisich: None. **S. Nawy:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.10/UU8

Topic: D.04. Vision

Support: NIH Grant F32EY022543

Title: ipRGCs mediate ipsilateral pupil constriction

Authors: *T. M. SCHMIDT[1], A. C. RUPP[1], K. S. CHEW[1], B. YUNGHER[3], K. K. PARK[3], S. HATTAR[2];
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Abstract: A recent finding demonstrated a larger constriction in the direct versus consensual pupillary light reflex (PLR) across several light intensities. In explanation, the authors provided evidence that the mammalian iris is directly photosensitive and requires the photopigment melanopsin, known for rendering a subset of retinal ganglion cells (RGCs) intrinsically photosensitive (ipRGCs). This exciting discovery challenged the dogma that the mammalian retina, and, specifically, ipRGCs are the only contributors to PLR. Given the identification of a novel pupil pathway, we sought to determine the relative contribution of ipRGCs and the iris muscle itself to the direct PLR. To achieve this, we performed optic nerve crush to kill RGCs (including ipRGCs) and study the intrinsic iris muscle PLR in isolation. We were surprised to find that the direct PLR was abolished 8 weeks following injury, suggesting that RGCs are necessary for this reflex. To correlate RGC death with the lack of pupil constriction, we measured the PLR for the first week following optic nerve crush and found that some constriction persisted in the initial days following injury and then declined as RGCs began to die. This indicates that RGCs are sufficient to drive the PLR independent of any brain circuitry. In support of these findings, we found that neuronal firing is critical for the PLR, as tetrodotoxin abolished pupil constriction except for a small response at very bright light intensities. Given these findings, we examined whether ipRGCs could directly mediate constriction via a direct projection from the retina to the iris muscle. In support of a role for ipRGCs in the direct PLR, we observed genetically labeled ipRGC axons coursing through both the dilator and constrictor muscles of the iris. We also found that although the consensual PLR is essentially lost in animals lacking the ipRGCs that project to pupil centers in the brain, the direct pupil constriction persists. In these animals, 200 ipRGCs remain, and they only project to circadian brain centers. Combined, these results indicate that the 200 ipRGCs also project to the iris to drive the direct PLR. Importantly, ipRGCs do not drive the PLR as motor neurons, but instead modulate acetylcholine release from parasympathetic fibers, as we find that atropine (a metabotropic acetylecholine receptor antagonist) application completely blocks direct pupil constriction. Collectively, these results demonstrate that ipRGCs project to the iris muscle directly, where they are sufficient to drive pupil constriction independent of the central pupil pathway or of any intrinsic photosensitivity of the iris muscle.

Disclosures: T.M. Schmidt: None. **A.C. Rupp:** None. **K.S. Chew:** None. **B. Yungher:** None. **K.K. Park:** None. **S. Hattar:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.11/UU9

Topic: D.04. Vision

Support: Wellcome Trust Grant 090684/Z/09/Z

Title: Novel light response mutants identified in a pupillometry screen of ENU mutant mice

Authors: *G. BANKS[1], T. OSBORN[2], C. A. POTHECARY[3], S. N. PEIRSON[3], R. G. FOSTER[3], P. M. NOLAN[1];
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Abstract: Non visual light response pathways in the eye and brain are key components in the regulation of sleep and circadian rhythms. Recent evidence has also implicated such pathways in mood and the pathology of depression. In order to better study and understand such processes we are performing a screen for mutations in light response pathways. To facilitate this we are using the pupillary light response (PLR) as a screening tool. The PLR is a non-visual response to light during which the iris of the eye constricts in response to illumination causing a decrease in pupil diameter. In our screening protocol mice are positioned so as to expose one eye to a light source while the contralateral eye is imaged, allowing us to track the PLR.
Our initial baseline studies have demonstrated strain related differences in the PLR. As expected mice carrying the *rd1* mutation have a reduced PLR compared to other strains. However *rd1* mutants also show an age related decrease in the PLR not found in non-*rd* mice. Interestingly mice carrying the *rd8* mutation did not show significant defects in the PLR but did also show an age related decrease in the PLR not observed in non-*rd* mice. Furthermore aged *rd8* mutant mice also showed a faster post illumination recovery compared to all other strains, including *rd1* mutants.
We have also begun a pupillometry screen of both F1 progeny and G3 pedigrees from ENU mutagenized male mice maintained at MRC Harwell. Thus far this screen has identified two mutant mouse lines characterised by defects in the PLR. Our PUPIL1 mouse line shows a mild reduction in PLR similar to that observed in melanopsin knockout mice. In contrast the PUPIL2 mutant line shows a severe reduction in PLR more similar to that observed in the *Trpm1* knockout line. We have mapped the PUPIL2 mutation to chromosome 13 and further genetic mapping is currently underway to enable us to identify the causative mutation. Once the causative mutation has been identified further characterisation will be performed at a

behavioural, histological and molecular level.
We hope that our data demonstrates that the PLR can be utilized as an informative, non-invasive, high throughput screening technique.

Disclosures: G. Banks: None. **T. Osborn:** None. **C.A. Pothecary:** None. **S.N. Peirson:** None. **R.G. Foster:** None. **P.M. Nolan:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.12/UU10

Topic: D.04. Vision

Support: Ministry of Knowledge Economy ,Republic of Korea 10030064

Title: Intravitral engraftment of mesenchymal stem cells loading on hydrogel in the ischemic rat retina

Authors: *J. LEE[1], J.-M. SHIN[1], S.-S. PAIK[1], C. YEUM[2], G. CHAE[2], I.-B. KIM[1], M.-H. CHUN[1], S.-J. OH[1];
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Abstract: Endeavor for development of a brand-new therapeutic strategy is also engaged for treating incurable neural retinal diseases. For this establishment, mesenchymal stem cells (MSCs) transplantation into the retina is ceaselessly tried and various techniques were designed. We aimed to test a biodegradable hyaluronic acid (HyA)-based hydrogel as a stem cell carrier, in order to upgrade the migratory potential of MSCs using a rat model of retinal ischemia-reperfusion injury (IR). IR model was made by a transient and extreme elevation of intraocular pressure via raising hydrostatic pressure value of 120 mmHg for 1 hour through a needle cannulation into the anterior chamber of the one side eyeballs of Sprague-Dawley rats. At 1 week post IR, DiI-labeled MSCs of three passages loaded on HyA-based hydrogel were applied on a dose of 1×10^5 cells/10 μl into the vitreous body of both IR and contra-lateral control eyeballs. HyA-based hydrogels were prepared with two different pH modulations. As a control for MSC carrier, 0.01 M phosphate buffered saline (pH 7.2, PBS) was used. MSC application was reserved for 3 weeks. MSCs localization and morphological alteration were accessed by immunohistochemistry and the visual function. The whole-mount retinas of all experiments were processed for anti-GFAP and anti-Iba1 immunohistochemistry and retinal sectional preparations were processed for anti-GFAP, -NGF, - BDNF, and -glutamine synthetase (GS)

immunohistochemistry. Among them, MSCs were much more accumulated in the border between the inner limiting membrane and the vitreous body loaded on HyA-based hydrogels than on PBS. Basic pH hydrogel showed higher localization than neutral pH one. All these antisera immunoreactivity appear in the Mueller cells and astrocyte in IR and differential downregulation after MSC application hydrogel treatment. Upon MSCs application on HyA-hydrogels, the ERG responses were significantly increased compared IR rats, more apparently in scotopic stimulation than in the photopic condition. These findings demonstrate that biodegradable HyA-based hydrogel is excellent for MSC localization in MSC transplantation into damaged retina and the MSCs delivered on those exert influence to some extent on functional reparation.
This study was supported by the Ministry of Knowledge Economy, Republic of Korea (10030064).

Disclosures: **J. Lee:** A. Employment/Salary (full or part-time):; full. **J. Shin:** A. Employment/Salary (full or part-time):; student. **S. Paik:** A. Employment/Salary (full or part-time):; full. **C. Yeum:** A. Employment/Salary (full or part-time):; full. **G. Chae:** A. Employment/Salary (full or part-time):; full. **I. Kim:** A. Employment/Salary (full or part-time):; full. **M. Chun:** A. Employment/Salary (full or part-time):; full. **S. Oh:** A. Employment/Salary (full or part-time):; full.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.13/UU11

Topic: D.04. Vision

Support: Lundbeck

Title: P2X7 receptor activation modulates light-evoked retinal ganglion cell synaptic responses and microglial morphology

Authors: *S. CHAVDA[1], P. J. LUTHERT[2,3], T. E. SALT[1];
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Abstract: ATP-gated P2X7 receptors (P2X7Rs) are expressed by various retinal cell types, including retinal ganglion cells (RGCs) and microglia, and are known to modulate glutamatergic neurotransmission in the central nervous system (CNS). P2X7Rs are also associated with the induction of microglia-derived cytokine release and neuronal injury, phenomena which are

common to many neuroinflammatory conditions in the retina and CNS. However, the role of P2X7Rs in the modulation of retinal synaptic function remains elusive. Using a dark-adapted, ex vivo mouse retinal wholemount preparation, the present study aimed to characterise the effect of P2X7R activation on light-evoked NMDA receptor (NMDAR)-mediated RGC synaptic responses, and on microglial morphology.
ON and OFF field excitatory post-synaptic potentials (fEPSPs) were recorded from the ganglion cell layer of acutely isolated retinal wholemounts (adult, male C57BL/6 mice) in response to a light stimulus (1s-duration LED flash, repeated every 3s). The NMDA receptor-mediated component of the responses was pharmacologically isolated with a Mg2+-free Krebs medium containing NBQX, picrotoxin, strychnine and tetrodotoxin). Additional drugs were applied to the bathing medium. Retinae were stained for microglia (α-IBA-1/IgG-Alexa488) and visualised with confocal microscopy.
The mGluR6 agonist, L-AP4 induced a reversible depression of the ON but not the OFF RGC fEPSP, consistent with mGluR6 activation at the photoreceptor-ON bipolar cell synapse. The NMDAR antagonist, D-AP5 reduced both ON and OFF RGC fEPSPs, confirming that the responses were mediated through NMDARs. BzATP (300μM), a P2X receptor agonist, depressed the ON (78±2% of control; $P<0.05$) but not the OFF RGC fEPSP peak amplitude. The effect of BzATP on the ON RGC fEPSP was reduced by the selective P2X7R antagonist, A438079 (10μM; 31±2% reduction; $P<0.05$) but not by suramin (10μM), a non-selective P2X receptor antagonist (ineffective at P2X7Rs at the dose tested), consistent with P2X7R activation. Additionally, adenosine (300μM) potentiated the ON response (110±2% of control), ruling out a possible effect of Bz-adenosine (a catabolic product of BzATP) on P1 receptors. Furthermore, BzATP caused an increase in microglial arbour area under similar conditions.
It is proposed that P2X7R activation depresses ON RGC glutamatergic synaptic transmission, as well as inducing changes in microglial morphology. Since P2X7Rs are well known for their function in microglia-derived cytokine release, this could also have implications for neural-immune interactions in the retina and CNS.

Disclosures: S. Chavda: None. **P.J. Luthert:** None. **T.E. Salt:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.14/UU12

Topic: D.04. Vision

Support: FONDECYT 1120513

Millennium Institute CINV

CONICYT AT-24121445

Title: Nitric oxide modulates OFF bipolar cell responses in the retina

Authors: *O. SCHMACHTENBERG, A. H. VIELMA;
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Abstract: Nitric oxide (NO) is involved in retinal signal processing, but its cellular actions are only partly understood. The main source of retinal NO is a subset of amacrine cells, which signal onto ON bipolar, amacrine and ganglion cells. Previous studies showed that NO inhibits ganglion cell OFF responses, but while NO is an established modulator of ON bipolar cells, little is known about its effects on OFF bipolar cells. We investigated if NO regulates glutamate responses in OFF bipolar cells through the activation of the soluble guanylyl cyclase-cGMP pathway. To this end, OFF bipolar cells were recorded in vibratome sections of rat retina with whole-cell patch clamp and identified by morphological and electrophysiological criteria. The cells were stimulated with a brief puff (1 s) of glutamate in the outer plexiform layer, while their axonal arbors in the inner plexiform layer were superfused with a long (10 s) puff of NO donors (NOC-12 and SNP) or the cGMP analogue 8-bromo-cGMP. Both NOC-12 and 8-bromo-cGMP eliminated the slow component of the response to glutamate, while the amplitude of the fast component remained unchanged. Addition of the soluble guanylyl cyclase inhibitor ODQ to the intracellular solution prevented this effect, suggesting a mechanism dependent on cGMP. Perfusion with the NO synthase inhibitors 7-NI and L-NNA prolonged the timing of the glutamate response, which could be reversed by the NO donors. These results show that NO alters the glutamate response characteristics of OFF bipolar cells, and confirm that the retinal OFF pathway is modulated by NO.

Disclosures: O. Schmachtenberg: None. **A.H. Vielma:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.15/UU13

Topic: D.04. Vision

Support: Damon Runyon Foundation

NIH Grant AG032322

Title: LKB1 and AMPK regulate synaptic remodeling in old age

Authors: *M. A. SAMUEL[1], P. E. VOINESCU[1], B. N. LILLEY[1], R. DE CABO[2], M. FORETZ[3], B. VIOLLET[3], D. G. VAVVAS[4], J. R. SANES[1];
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Abstract: Age-related declines in neural function result in part from alterations in synapses. Unfortunately, very little is known about the molecular pathways that underlie these changes in part because most age-related synaptic alterations are subtle and difficult to quantify, owing to the small size of the synapses and the complexity of brain circuitry. We therefore focused on a large, easily-visualized synapse in the outer retina that undergoes dramatic alterations in old mice and humans. In old retina, numerous ectopic synapses develop in the photoreceptor layer along aberrant processes arising from horizontal and bipolar cell neurons. We show that defects in the LKB1 and AMPK energy regulatory pathway underlie these alterations. In old animals, synaptic remodeling is accompanied by specific decreases in the levels of both LKB1 and active AMPK (phosphoT172-AMPK), while the levels and phosphorylation state of other LKB1 targets are unaffected. In the absence of either LKB1 or AMPK, young mice develop retinal defects similar to those that occur in old wild type animals. Using conditional alleles, we examined the cellular basis of this remodeling and show that LKB1 and AMPK function specifically in photoreceptors to directly modulate rod axon localization. Moreover, restoring AMPK genetically in either old or LKB1-deficient animals rescues the synaptic alterations. Pharmacological (metformin) or dietary (caloric restriction) interventions that increase AMPK activity also attenuate age-related synaptic changes. Conversely, alterations are exacerbated in mice fed a high-fat diet. Together, these results identify a molecular cause of age-related synaptic remodeling and suggest that energy homeostasis pathways play a critical role in preserving neural function in old age.

Disclosures: M.A. Samuel: None. **P.E. Voinescu:** None. **B.N. Lilley:** None. **R. de Cabo:** None. **M. Foretz:** None. **B. Viollet:** None. **D.G. Vavvas:** None. **J.R. Sanes:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.16/UU14

Topic: D.04. Vision

Support: NINDS IRP: NS002986

Title: Inhibition controls the occupancy of the readily releasable pool of vesicles at the rod bipolar synapse to normalize contrast coding

Authors: *N. W. OESCH, J. S. DIAMOND;
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Abstract: The nervous system often encodes changes in signal level, rather than absolute level allowing for efficient coding of inputs over a large range. In the visual system, where this challenge is particularly acute, the measure of difference between luminance values is referred to as contrast. Recently, we demonstrated that the excitatory rod bipolar cell (RBC) synapse in the retina both computes contrast and relays luminance information in its synaptic output, and we demonstrated that contrast is calculated by stepping between different steady-state levels of readily releasable pool occupancy. This mechanism, in isolation generates a contrast signal that varies with background, yet our perception of contrast remains constant across the entire range of vision. Here we demonstrate that synaptic inhibition onto the RBC axon/synaptic terminal influences the occupancy of the readily releasable pool of vesicles at the RBC synapse to both normalize the contrast gain as well as extend the range of backgrounds over which both contrast and luminance can be coded by RBC synaptic output.

Disclosures: N.W. Oesch: None. **J.S. Diamond:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.17/UU15

Topic: D.04. Vision

Support: FFB

CIHR

Title: Multiple layers of inhibition to DSGCs are differentially modified by light

Authors: *A. HOGGARTH, S. TRENHOLM, A. MCLAUGHLIN, G. AWATRAMANI;
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Abstract: Introduction
Lateral inhibition mediated by horizontal/amacrine cells is known to be down-modulated in dark-adapted conditions (Barlow et al., 1956), leading to a loss of the inhibitory surround receptive fields of retinal ganglion cells. If and how ganglion cells can maintain their computational ability

in the face of such changes in their receptive filed organization is not clear. Here, we examine the impact of background light levels on the response properties of directionally selective ganglion cells (DSGCs), which rely heavily on inhibition for their ability to code direction.
Methods
DSGCs identified in the Hb9::eGFP transgenic mouse retina were targeted for whole-cell and extracellular patch clamp recordings, using 2-photon laser scanning microscopy. Mice were dark adapted for a minimum of 2 hours prior to experimentation. Responses to stationary or moving (in eight directions) spot stimuli of increasing sizes centered over the soma were measured at different background light levels that straddled the rod and cone thresholds (10-2 R*/s to 103 R*/s).
Results
In light-adapted conditions, small spots (~200 um) evoked stronger responses in DSGCs, compared to larger spots (1000 um; <50%), indicating the presence of strong inhibitory surround. Under dark-adapted conditions, this inhibitory surround essentially disappeared. Despite these changes in surround inhibition, responses to moving stimuli remained DS. To understand how DS could be maintained despite the loss of inhibition to the circuit we measured inhibitory (Vhold 0 mV) and excitatory (Vhold -60 mV) current in voltage-clamped DSGCs. Interestingly, we found that regardless of the adaptational state, the strength of direct excitation and inhibition to DSGCs scaled in parallel. In light-adapted retina, both inhibition and excitation decreased when the spot size was increased above 200 um, while in the dark-adapted state they increased as a function of spot size. These results indicated that surround inhibition co-modulates direct excitation and inhibition, at a level presynaptic to the DSGC. In addition, we find that the balanced changes in excitation and inhibition permitted conventional null direction inhibition (inhibition evoked by stimuli moving in the null direction) to veto excitation across all light levels.
Conclusions
Light levels appear to selectively modulate wide-field inhibition that functions to co-modulate local excitation and inhibition to DSGCs, allowing them to code the same direction over a large range of light intensities with distinct spatiotemporal characteristics.

Disclosures: A. Hoggarth: None. **S. Trenholm:** None. **A. McLaughlin:** None. **G. Awatramani:** None.

Poster

553. **Retinal Circuitry: Synaptic Interactions**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 553.18/UU16

Topic: D.04. Vision

Support: T32 NS007220-27

Title: PlexinA4 restricts retinal ganglion cell axon arborization in the superior colliculus

Authors: *S. G. THAKAR, Y. ZOU;
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Abstract: Precise connections between retinal ganglion cells and their brain targets, such as the superior colliculus (SC), are essential for visual system function. Axon terminal arbors of different retinal ganglion cells (RGCs) assume characteristic columnar and laminar specific patterns in the SC, which allows them to synapse on specific cell types. Based on the expression patterns of semaphorin ligands and receptors, we tested the role of a semaphorin receptor component, PlexinA4, in controlling RGC axon targeting. The *Calretinin-eGFP* (*CB2-GFP*) transgenic mice selectively express GFP in transient OFF-RGC, which target the deeper retinorecipient layer of the SC (lower stratum griseum superficialis, lSGS). *CB2-GFP* mice were crossed into the *PlexinA4* knockout line to evaluate OFF-RGC axon pathfinding and targeting. We find that *PlexinA4*$^{-/-}$ RGC axons grow and steer normally down the optic nerve and find their appropriate overall targets, the lateral geniculate nucleus and SC, and within those targets, *PlexinA4*$^{-/-}$ RGC axons do not display topographic mapping defects. However, *PlexinA4*$^{-/-}$ RGC axons did exhibit severe defects in the laminar location of their axon arbors in the SC. In *CB2*$^{+}$*:PlexinA4*$^{-/-}$, and to a lesser degree *CB2*$^{+}$*:PlexinA4*$^{+/-}$, the OFF-RGC axon arbors invade the more superficial layer of the SC (upper stratum griseum superficialis) rather than remain restricted to the lSGS as they do in *wild-type* mice. These data indicate PlexinA4 is required for normal layer specific targeting of OFF-RGC axons in the SC, and suggest that, like for RGC dendrites, repellant mechanisms may serve to restrict RGC axon arbors within their target region.

Disclosures: S.G. Thakar: None. **Y. Zou:** None.**Poster**

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.01/UU17

Topic: D.04. Vision

Support: NIMH00022

Title: The neural ensemble recording in the visual cortex of awake mouse

Authors: *X. LI, H. ZHANG, J. TSIEN;
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Abstract: The development of genetic tools for analyzing neural circuits promoted the mouse to be a potential powerful model for studying the neural mechanism of cognition and behavior. In order to study visual recognition, we investigated the mouse visual cortex in awake condition and did neural ensemble recording. The first step we trained the mouse to keep sitting quietly with seldom eye movement, this helped us to investigate the basic characters of the visual neurons. Equiped with the drivable microelectrode arrays which could be fixed on mouse head for chronic recording, about 50 firing units could be obtained simultaneously with one 64 channels array in stereotrode mode. In the primary visual cortex of awake mouse, we found that the receptive fields of mouse visual neurons were very large and with long response latency. The receptive fields of neurons belonging to a local range (D = 0.8 mm) were in non- uniform distribution. Most neurons had strong orientation and spatial frequency selectivity. Less than half had strong temporal and direction selectivity. These suggested that more mouse V1 neurons could tell shape than motion. The neurons with similar response characters (preferred orientation, simple or complex cell) were not clustered, but in a salt and pepper arrangement.

Disclosures: X. Li: None. **H. Zhang:** None. **J. Tsien:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.02/UU18

Topic: D.04. Vision

Title: Differential wakening effects on inhibitory and excitatory neurons in visual cortex, revealed by *In vivo* two-photon functional imaging

Authors: *R. KIMURA[1], K. SOHYA[1], M.-S. SAFARI[1], T. EBINA[1], Y. YANAGAWA[2], T. TSUMOTO[1];
[1]RIKEN BSI, Wako, Saitama, Japan; [2]Gunma Univ. Grad. Sch. of Med., Maebashi, Japan

Abstract: In attempts to understand neural circuit mechanisms underlying visual perception and cognition, most of the previous works were carried out in anesthetized animals. To fully understand such neural mechanisms, however, it is necessary to record and analyze activities of visual cortical neurons in awakening animals. Also it is important to identify recorded neurons as inhibitory or excitatory because inhibitory interneurons play characteristic, specified roles in the operation of neuronal circuits in the visual cortex.

To address these issues, we directly compared visual response properties of the same cortical neurons between in the awakening and anesthetized states by applying the two-photon calcium imaging method to the transgenic rodents, in which inhibitory and excitatory neurons are identified in vivo. Functional imaging of cortical neurons was carried out by injecting a calcium indicator (fura 2-AM) and an astrocyte marker (SR101) into layer 2/3 of the primary visual cortex of adult VGAT-Venus transgenic rats, in which GABAergic neurons were identified with Venus fluorescence. Comparing the visual responses between the two states (monitored by EEG activity), we found that GABAergic neurons increased the magnitude of visual responses whereas excitatory neurons did not. Furthermore the decay time of excitatory neurons became faster so that their visual responses were shorter-lasting in the awake state than in the anesthetized state.
Previously it was suggested that acetylcholine (ACh) may be involved in the brain state-dependent changes in activities of cortical neurons through the basal forebrain projection from the nucleus basalis (NB). However, it is not clear whether this cholinergic modulation operates in a differentiated manner between GABAergic and excitatory neurons. To address this issue, we activated NB neurons through the optogenetic method in addition to the conventional electrical stimulation method. The changes in visual responses induced by activation of NB were compared with the results obtained with awakening animals.

Disclosures: R. Kimura: None. **K. Sohya:** None. **M. Safari:** None. **T. Ebina:** None. **Y. Yanagawa:** None. **T. Tsumoto:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.03/UU19-DP7

Topic: D.04. Vision

Support: Paul Allen

Allen Institute for Brain Science

Title: Cell-type specific organization of functional circuits in mouse visual cortex

Authors: *H. ZENG, L. LI;
Allen Inst. For Brain Sci., SEATTLE, WA

Abstract: Cortex consists of many types of neurons with various morphological, physiological and genetic properties, but the cell-type specific role in cortical (micro)circuit construction and

neural information computation remains largely unknown. Morphological, physiological and genetic properties of different types of neurons need to be characterized in order to address these questions. Along with others at the Allen Institute for Brain Science, we have set up a Cell Types program aimed at understanding these properties of genetically defined cell types in mouse visual system. To reveal how different types of neurons are inter-connected and assembled into microcircuits for neural information processing, we are employing two-photon targeted whole-cell recording in vivo in transgenic mice in which individual types of neurons are fluorescently labeled. We will characterize physiological properties of neuronal types and integrate data with information of gene expression patterns and morphology generated from separate efforts at the Allen Institute.

Disclosures: H. Zeng: None. **L. Li:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.04/UU20

Topic: D.04. Vision

Support: NSERC grant 194670

Title: Functional maps in V1 of D1 and D2 dopamine receptors knockout mice

Authors: *B. O. SOUZA[1], S. THOMAS[1], J. M. BEAULIEU[2], C. CASANOVA[1];
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Abstract: Dopamine (DA) is a neurotransmitter that plays an important role in the processing of the visual information in the retina. In addition to their implication in retinal function, DAergic receptors are also found in the visual cortex. However, the functional impact of these receptors on the structure and function of the visual cortex remains poorly understood. The aim of the present study is to determine the impact of the deletion of dopamine D1 and D2 receptors (D1-KO and D2-KO) on cortical organization and visual processing in V1. Adult mutant and wild-type mice (D1R-KO : n=2, D1R-WT : n=3, D2R-KO : n=3, D2R-WT : n=3) were placed in a stereotaxic apparatus under urethane anesthesia (2g/Kg). Activity in V1 was measured by optical imaging of intrinsic signals under a 630 nm illumination. Using a periodic stimulation paradigm (Kalatsky and Stryker, 2003), visuotopic maps were analyzed to measure structural parameters such as V1 shape, cortical magnification factor, scatter, binocularity and ocular dominance. Contrast sensitivity, spatial frequency selectivity and cortical acuity were evaluated using

dynamic sinusoidal gratings of different contrasts (6, 12, 25, 50 and 100% of contrast at 0.02 cpd) or spatial frequencies (0.005, 0.01, 0.02, 0.04, 0.8, 0.16, 0.32, 0.64 cpd at 100% contrast). Contrast sensitivity was reduced in both D1 and D2-KO mice. Indeed, the contrast value necessary to induce 50 % of the maximal response was increased by 70 % for D1-KO ($p<0.01$) and 55 % for D2-KO mice ($p<0.05$) when compared to their WT littermates. Furthermore, our preliminary results suggest a decreased cortical acuity for D1-KO compared to control littermates (0.09 ± 0.002 cpd vs 0.14 ± 0.002 cpd). This effect was not observed in D2-KO mice. Surprisingly, the various structural parameters extracted from V1 visuotopic maps were unaffected by the loss of expression of either D1 or D2 receptors. In conclusion, our preliminary data indicate that contrast sensitivity was reduced in both D1-KO and D2-KO mice. Our data also suggest that visual acuity is modulated by DA, through a D1R-specific signaling pathway. The lack of structural alteration in V1 maps of D1 and D2-KO mice suggests that DA receptors do not play a crucial role in the development of the mouse primary visual cortex.

Disclosures: B.O. Souza: None. **S. Thomas:** None. **J.M. Beaulieu:** None. **C. Casanova:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.05/UU21

Topic: D.04. Vision

Support: CREST

Title: Functional organization of clonally related neurons in mouse visual cortex during development

Authors: *G. OHTSUKI[1], C. LOIS[2], K. OHKI[1];
[1]Mol. Physiol., Kyushu University, Grad Sch. of Med., Fukuoka, Japan; [2]Neurobio., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Recently, it was revealed that orientation preference among clonally related cells is more similar than among unrelated cells (Li et al., 2012; Ohtsuki et al., 2012). However, not all clonally related cells share response selectivity in adult mice (P49-62), suggesting that cell lineage is not the only determinant of response selectivity (Ohtsuki et al., 2012). In this study, we tested three hypotheses. First, the similarity of preferred orientation between clonal neurons may be strong just after eye opening (Li et al., 2012) and may be reduced in adult in the course of maturation of the cortical circuit. Second, the vertically aligned clonal cells in the cortex may have higher similarity in orientation preference. Third, the functional similarity of clonal cells

may be moderate from the eye opening to matured state, and it may be moderate even among vertically aligned clonal cells. By using the combination of *in vivo* two-photon Ca^{2+} imaging and a transgenic mouse which labels all the progeny derived from single cortical progenitor cells, we examined orientation, direction and spatial frequency selectivity of clonal and other cells at time points of the eye opening, 3-10 days later and matured. We also examined whether functional similarity among clonally related cells depends on the positional relation among those cells in each developmental stage, and analyzed proportion of difference in preferred orientation angle in vertically aligned cell population among all cells or clonal sisters.

Disclosures: G. Ohtsuki: None. **C. Lois:** None. **K. Ohki:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.06/UU22

Topic: D.04. Vision

Support: HHMI

Kavli Institute for Brain Science

NEI

NINDS

NIHM

Keck Foundation

the U. S. Army Research Office

Title: Spontaneous neuronal ensemble evoked by visual stimulation in awake mice

Authors: *J.-E. MILLER, I. AYZENSHTAT, R. YUSTE;
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Abstract: The cortical microcircuit is dominated by recurrent excitatory connections, and it has been long suggested that this could enable ongoing reverberating activity. To better understand the dynamics of neuronal activity present in neocortex, we performed two-photon calcium imaging of populations of layer 2/3 cortical neurons in mouse primary visual cortex in vivo in

anesthetized and awake mice, positioned on a floating trackball. We characterized the spatiotemporal dynamics of cortical activity in the presence or absence of visual inputs, using either drifting gratings or a natural movie to evoke population activity or a black screen to measure spontaneous activity.
We find that, under all conditions (gratings, natural movie, and spontaneous), most neurons become active as part of an ensemble, i.e. a group of neurons responding together forming coactive spatial patterns. There appears no appreciable difference in coactive pattern properties or dynamics according to different brain states (i.e. anesthetized or awake) or stimulus conditions. Different coactivations repeat significantly, involving many of the same neurons, although coactive groups are flexible and the same neurons can be part of different coactivations. Repeated coactive patterns are more frequent in awake animals although they are also present under anesthesia. Finally, the spontaneous coactive groups of neurons can be triggered also by specific visual stimulation. These overlapped coactive patterns during both spontaneous and visually evoked activities are statistically significant in awake mice, but not in anesthetized ones. Our results indicate that cortical activity mostly occurs in coactive patterns, and that, in the awake brain, sensory evoked activity is built out of a vocabulary of patterns that are already present in the spontaneous ongoing activity. These coactive patterns could represent emergent functional circuit states.

Disclosures: J. Miller: None. **I. Ayzenshtat:** None. **R. Yuste:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.07/UU23

Topic: D.04. Vision

Support: HHMI

Gatsby California Circuits Consortium

Title: Receptive fields and orientation tuning of thalamic excitation onto single neurons of the mouse's visual cortex

Authors: *A. D. LIEN[1], M. SCANZIANI[2];
[1]UCSD, La Jolla, CA; [2]UCSD, HHMI, La Jolla, CA

Abstract: A fundamental feature of cortical organization is that sensory information reaches the neocortex via excitatory synaptic input from the thalamus. Yet the specific sensory information

represented by the thalamic excitation received by a single cortical neuron are unknown. This is due to the difficulty in distinguishing thalamic excitation from that produced by intracortical excitatory inputs. Here we isolate thalamic excitation onto mouse L4 visual cortical neurons by performing in vivo whole-cell recordings while silencing intracortical excitation via optogenetic activation of inhibitory interneurons. We mapped the ON and OFF receptive fields of thalamic excitation using small black or white squares presented at various locations in the mouse's field of view. We find that the receptive field of thalamic excitation is organized into overlapping ON and OFF subfields with spatially offset peaks. The offset between ON and OFF subfields is a potential mechanism by which thalamic excitation can be selective for the orientation of a stimulus in visual space. Consistent with this hypothesis, we find that in response to drifting gratings, the F1 modulation (or peak) of thalamic excitation is tuned to the orientation of the grating. Furthermore, the relative position of the ON and OFF peaks is predictive of the preferred orientation. In contrast, the total excitatory charge (or average excitation) across the grating duration is poorly tuned, suggesting that recently described orientation and direction-selective thalamic neurons do not contribute to the tuning of thalamic excitation onto L4 neurons. In conclusion, we demonstrate that thalamic excitation is tuned for orientation and that its receptive field structure, namely the separation of ON and OFF subfields in visual space, likely forms the basis for this tuning.

Disclosures: A.D. Lien: None. **M. Scanziani:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.08/UU24

Topic: D.04. Vision

Support: EMBO LTF 1114-2009

Jane Coffin Childs Memorial Fund for Medical Research

NIH NS047101

NARSAD

HHMI

Gatsby charitable foundation

Title: Inhibition of inhibition in visual cortex: The logic of connections between molecularly distinct gabaergic neurons

Authors: *C. PFEFFER[1,2], M. XUE[1], M. HE[3], Z. HUANG[3], M. SCANZIANI[1,2];
[1]Div. of Biol. Sci., UCSD, La Jolla, CA; [2]HHMI, La Jolla, CA; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Cortical inhibitory neurons contact each other to form a network of inhibitory synaptic connections. Our knowledge of the connectivity pattern underlying this inhibitory network is, however, still incomplete. Here we address the interaction between the three largest classes of molecularly distinct inhibitory neurons in mouse visual cortex: the Parvalbumin (PV), the Somatostatin (SOM) and the Vasoactive Intestinal Peptide (VIP) expressing inhibitory neurons. We use conditional expression of Channelhodopsin in specific Cre-driver lines to photoactivate the three distinct classes of inhibitory neurons in acute cortical slices. We record the postsynaptic inhibition in GABAergic interneurons which we identify post-hoc using single-cell rtPCR expression profiling. We discover a simple and complementary interaction scheme between the inhibitory neuron populations. PV expressing interneurons strongly inhibit one another but, surprisingly, provide little inhibition to other populations. In contrast, SOM expressing interneurons avoid inhibiting one another, yet strongly inhibit all other populations. Finally, VIP expressing interneurons preferentially inhibit SOM interneurons. This scheme occurs in supra- and infra-granular layers, suggesting that inhibitory networks operate similarly at the input and output of visual cortex. Thus, as the specificity of connections between excitatory neurons forms the basis for the cortical canonical circuit, the scheme described here outlines a standard connectivity pattern among cortical inhibitory neurons.

Disclosures: C. Pfeffer: A. Employment/Salary (full or part-time):; HHMI/UCSD. **M. Xue:** A. Employment/Salary (full or part-time):; UCSD. **M. He:** A. Employment/Salary (full or part-time):; Cold Spring Harbor Laboratory. **Z. Huang:** A. Employment/Salary (full or part-time):; Cold Spring Harbor Laboratory. **M. Scanziani:** A. Employment/Salary (full or part-time):; HHMI/UCSD.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.09/UU25

Topic: D.04. Vision

Support: NSF GRFP

Title: Role of thalamocortical input to mouse primary visual cortex in maintenance of excited states

Authors: *K. REINHOLD, M. SCANZIANI;
Neurosciences, Univ. of California At San Diego, San Diego, CA

Abstract: The principal feedforward sensory input to the primary visual cortex (V1) is the thalamus, yet thalamocortical synapses comprise a small fraction of the total synapses in V1. Local recurrent circuits account for the majority of these synapses, potentially supporting cortical activity in the absence of ongoing feedforward drive from the thalamus. For how long can a visually evoked representation in V1 last without this ongoing feedforward drive? To test this, we require a way to rapidly and reversibly remove thalamic input to the neocortex *in vivo* and measure the effects on an ongoing cortical representation. We have developed an optogenetic method to do this in the visual system of mice by activating inhibitory inputs from the GABAergic reticular thalamic nucleus onto relay cells of the dorsal lateral geniculate (dLGN) and lateral posterior (LP) nuclei, while recording unit activity across layers of V1. Our optogenetic approach is able to silence the thalamus within a few milliseconds, enabling us to measure the time course of activity decay intrinsic to the V1 circuit. We find that a visually evoked response in V1 decays within 40 ms of removal of the feedforward thalamic input. We also discover that spontaneous activity under anesthesia does not require the thalamus, while spontaneous activity in the *awake* mouse includes a large thalamus-dependent component. Finally, we investigate circuit mechanisms underlying the ability of the cortex to precisely follow feedforward thalamic input yet sustain its own intrinsic activity in the absence of this feedforward drive. These results explain how V1 can encode changes in the external world with such a high temporal fidelity yet exhibit waves of spontaneous activity in certain states such as sleep.

Disclosures: K. Reinhold: None. **M. Scanziani:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.10/UU26

Topic: D.04. Vision

Support: Jane Coffin Childs Memorial Fund for Medical Research fellowship

HHMI

Gatsby Charitable Foundation

Title: Cell-type specific homeostatic mechanisms in mouse visual cortex

Authors: *M. XUE, M. SCANZIANI;
Div. of Biol. Sci., UCSD, La Jolla, CA

Abstract: The activity of cortical neurons critically depends on the relationship between the levels of synaptic excitation and inhibition that they receive. One prominent feature of the cortical synaptic organization is that the locally generated inhibition is proportional to the local or incoming excitation. This proportionality between excitation and inhibition is thought to be fundamental to the functional properties of cortical neurons such as receptive field structure, timing, gain, and so on. Disruption of the proper excitation and inhibition relationship often leads to neurological disorders. The mechanisms that establish the exact relationship between the two opposing synaptic conductances are, however, still poorly understood. Similarly, whether this relationship is individually regulated in each cortical neuron is unknown. We found that individual layer 2/3 pyramidal neurons in the mouse primary visual cortex exhibited highly heterogeneous spiking activity in vivo. Slice experiments showed those layer 2/3 pyramidal neurons that responded to visual stimuli more robustly in vivo received larger glutamatergic excitation from their main afferents, layer 4 projection neurons and concurrently larger GABAergic inhibition from parvalbumin-expressing (PV) cells than the ones that responded less robustly, thus maintaining the excitation to inhibition ratios across neurons despite their different firing rates. Furthermore, we found that suppression of layer 2/3 pyramidal neuron activity selectively decreased PV cell-mediated inhibition without affecting layer 4-mediated excitation. These results indicate that individual layer 2/3 pyramidal neurons autonomously adjust their levels of inhibition in an activity-dependent manner by selectively modulating PV cell-mediated inhibition, which provides a mechanism for cortical circuit to maintain the proper excitation to inhibition ratio at the single-neuron level.

Disclosures: M. Xue: None. **M. Scanziani:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.11/VV1

Topic: D.04. Vision

Support: HHMI

The Gatsby Charitable Foundation

Title: Neural circuits for the cortical control of the optokinetic reflex

Authors: *B. LIU, A. HUBERMAN, M. SCANZIANI;
UCSD, La Jolla, CA

Abstract: The optokinetic reflex (OKR) is an involuntary eye movement that allows the eyes to track a moving visual stimulus and thus to stabilize images on the retina. While mediated by the phylogenetically old accessory optic system (AOS) in the midbrain, interestingly, the performance of this innate behavior in mammals can also be modulated by the visual areas of the neocortex. However the circuit mechanisms by which the cortex exerts its modulatory roles on the OKR remain rather elusive. To investigate this question, we optogenetically silenced the mouse visual cortex during the OKR elicited by drifting gratings on a virtual drum and monitored by infrared video-oculography. We observed that transient cortical silencing significantly reduced the gain of the OKR by 10-30%, depending on visual stimulus parameters. Consistently, following the inactivation of the visual cortex, visually evoked activity in the AOS nucleus decreased. Moreover, with retrograde tracing and slice electrophysiology, we showed that a small portion of layer 5 neurons in the mouse visual cortex form functional synaptic connections onto AOS neurons. These results suggest that the visual cortex influences the OKR behavior, at least in part, through the direct corticofugal projection to the AOS system.

Disclosures: B. Liu: None. **A. Huberman:** None. **M. Scanziani:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.12/VV2

Topic: D.04. Vision

Support: Helen Hay Whitney Foundation Postdoctoral Fellowship

HHMI

Gatbsy Foundation

Title: A virtual foraging task for studying vision in mice

Authors: *S. R. OLSEN, M. SCANZIANI;
UCSD/HHMI, San Diego, CA

Abstract: Cognition and behavior are mediated by dynamic activity in cortical circuits. To study the mechanisms of cortical dynamics during behavior, we developed a paradigm in which mice use their vision to navigate along a linear track. In this task a mouse runs on a circular treadmill in order to move objects across a computer monitor. Over the course of several weeks of training, mice learn to collect rewards by running to and stopping on target objects, while running past unrewarded distractor objects. Using this paradigm we trained mice on an orientation discrimination task. Mice can learn to discriminate orientation differences less than 10 degrees. Optogenetic silencing of the visual cortex during the task severely disrupted performance, suggesting this behavior is cortex-dependent. In addition, the time course of learning and specificity of fine orientation discrimination is consistent with perceptual learning. This task (virtual foraging task) should be useful for studying various aspects of visual behavior ranging from stimulus detection/discrimination to spatial attention.

Disclosures: S.R. Olsen: None. **M. Scanziani:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.13/VV3

Topic: D.04. Vision

Support: National “973” Program

Chinese Academy of Sciences (CAS) “Hundred Talents Program” awards

Title: Topological organization of spatial frequency in mouse primary visual cortex

Authors: *X. ZHANG, X. AN, J. PENG, S. CAI, W. WANG, Y. YANG;
Lab. of Plasticity of Sensory Syst. Develop., Anhui, China

Abstract: Summary:
Visual acuity, reflected as spatial discrimination and represented in spatial frequency (SF) tuning of neurons in V1, is of particular importance for animal survival. Although columnar organization of neurons with similar response properties is a salient feature in mammalian V1, the functional organization of neurons in rodent V1 processing different SFs remains unknown. Here, by combining intrinsic optical imaging and single-unit recordings, we revealed the topological organization of SF tuning in mouse V1 to accommodate different spatial discrimination tasks in lower and upper visual space. Firstly, we found that in both monocular and binocular zones, neurons in the anterior cortical area representing lower visual field

preferred low SF, whereas neurons in the posterior part representing upper visual field were apt to possess high spatial acuity. Secondly, we showed that the cutoff SF maps changed in a continuous manner along vertical visual field. Thirdly, we demonstrated that this specific structured map emerged from eye opening and was preserved into adulthood. Finally, we established that although the development of visual acuity was delayed in dark-reared animals, the topological organization of SF maps was similar to that in normal reared animals, suggesting that the functional organization of SF preference is inherent and independent of visual experience. Taken together, our findings provide new insights into the origin of SF columns in higher mammalian, and underscore the significance of SF maps for mice survival by avoiding predators' attack from the upper space.

Disclosures: X. Zhang: None. **X. An:** None. **J. Peng:** None. **S. Cai:** None. **W. Wang:** None. **Y. Yang:** None.

Poster

554. **Striate Cortex: Functional Organization I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 554.14/VV4

Topic: D.04. Vision

Support: NIH NRSA T32-NS058280

EY016052

MH082935

Title: The assembly and function of PV microcircuits in the primary visual cortex

Authors: *A. BAOHAN[1], P. GOLSHANI[2], J. T. TRACHTENBERG[1];
[1]Dept. of Neurobio., [2]Dept. of Neurol., UCLA, Los Angeles, CA

Abstract: The emergent properties of cortical function are predicated on the dynamic and intricate balance of excitation and inhibition. Cortical inhibition is supplied by a diverse group of neurons with distinct structural, molecular, electrophysiological and likely, functional properties. Parvalbumin-expressing (PV), fast-spiking inhibitory neurons account for the majority of neocortical inhibitory neurons, yet little is known about how PV microcircuits are assembled and what function they serve in cortical computation. We find that phosphatase and tensin homolog (PTEN) regulates the assembly of neocortical PV-pyramidal microcircuits via the AKT signaling pathway. We generated a transgenic mouse with a PV-specific single copy deletion of *PTEN*

starting after the first postnatal week (PV-PTEN$^{+/-}$). Since PTEN negatively regulates the AKT signaling pathway, loss of PTEN activity results in increased AKT pathway activity. While these PV-PTEN$^{+/-}$ mice show normal cortical lamination and PV intrinsic properties, we find that they display an aberration in PV-pyramidal microcircuitry. Specifically, paired PV-pyramidal recordings in layer 2/3 of the primary visual cortex (V1) in acute slices reveal a significant unilateral decrease in PV to pyramidal connectivity, while reciprocal pyramidal to PV connections are preserved. The strength and short-term plasticity of the remaining PV to pyramidal connections are normal, which suggests that the decrease in PV to pyramidal connectivity is due to a decrease in the number of functional PV to pyramidal synapses, not a decrease in presynaptic GABA release probability. The 32% reduction in PV-mediated inhibition results in increased excitability of local pyramidal neurons, which cannot be attributed to intrinsic changes. We are currently working on interlaminar circuitry as well as intralaminar input-output transformation *in vivo* in awake mice with population Ca^{2+} imaging, using V1 as a model circuit. The elucidation of a novel role for the AKT signaling pathway in PV microcircuits may shed light on the mechanisms of neuropsychiatric disorders such as autism spectrum disorders, as many of the candidate genes for these disorders converge on the AKT signaling pathway.

Disclosures: A. Baohan: None. **P. Golshani:** None. **J.T. Trachtenberg:** None.**Poster**

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.01/VV5

Topic: D.04. Vision

Support: NIH Grant EY020692

NIH Grant EY017292

NIH Grant EY017921

Title: Phase-dependent coding in cortical ensembles during attentional tracking

Authors: *G. H. MULLIKEN[1], R. DESIMONE[2];
[1]McGovern Inst., MIT, CAMBRIDGE, MA; [2]McGovern Inst., MIT, Cambridge, MA

Abstract: We investigated how cortical neurons encode a moving object during attentional tracking. Monkeys were trained to covertly track a moving dot along an isoeccentric trajectory that passed through the receptive field (RF) of a neuron(s). Our analyses sought to characterize both signal encoding as well as noise suppression properties of V4 and LIP neurons by

quantifying the relationships between spike timing and the phase of the local field potential (LFP). We first evaluated the baseline state of neural activity. In the absence of a visual stimulus, a neuron's likelihood of firing was enhanced during the depolarized phase of the LFP (i.e., the trough) for low and high frequency bands of the LFP (e.g., theta, 4-6 Hz, and gamma, 30-70 Hz). This finding indicates that, by default, neuronal spiking correlates with depolarized net synaptic activity reflected in the extracellular LFP, irrespective of its constituent frequency. During stimulus presentation, we found that the probability of neurons to fire at the depolarized phase of the gamma-LFP was preserved, and even enhanced, while spike timing became desynchronized to the theta-LFP.
It has been suggested that the low frequency LFP may largely constitute a common mode noisy synaptic input into early visual processing areas, which is unrelated to a stimulus and interferes with neurons' ability to encode a signal. If true, then spikes generated during the peak of the theta-LFP should be less impacted by common-mode noise. We quantified a neuron's phase-dependent encoding strength by computing the mutual information between a neuronal spike train and the time-varying stimulus position for intervals coinciding with different phases of the theta LFP. Spikes that fired on the peak of the theta-LFP tended to encode more information about the stimulus position than spikes occurring during the trough of the theta-LFP. In addition, we assessed whether the theta-LFP phase might impact population coding by computing the pairwise spike count correlation between neurons with overlapping RFs. Interestingly, we found that pairwise correlations were also larger during the peak of the theta rhythm. In summary, these data provide evidence that neuronal populations suppress low-frequency common-mode noise, effectively reducing the in-band noise floor and enabling higher fidelity signal encoding of position. Lastly, the effects of attention on these phase-dependent coding schemes will also be presented.

Disclosures: G.H. Mulliken: None. **R. Desimone:** None.

Poster

555. Extrastriate Cortex: Neural Coding

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.02/VV6

Topic: D.04. Vision

Support: NEI Grant RO1-EY03164

NEI Grant K99-EY022116

Title: A two-stage cascade model of BOLD responses in human visual cortex

Authors: *K. N. KAY, J. WINAWER, A. ROKEM, A. MEZER, B. A. WANDELL;
Stanford Univ., Stanford, CA

Abstract: Visual neuroscientists have discovered fundamental properties of neural representation through careful analysis of responses to controlled stimuli. Typically, different properties are studied and modeled separately. To integrate our knowledge, it is necessary to build general models that begin with an input image and predict responses to a wide range of stimuli. In this study, we develop a model that accepts an arbitrary band-pass grayscale image as input and predicts blood oxygenation level dependent (BOLD) responses in early visual cortex as output. The model has a cascade architecture, consisting of two stages of linear and nonlinear operations. The first stage involves well-established computations--local oriented filters and divisive normalization--whereas the second stage involves novel computations--compressive spatial summation (a form of normalization) and a variance-like nonlinearity that generates selectivity for second-order contrast. The parameters of the model, which are estimated from BOLD data, vary systematically across visual field maps: compared to primary visual cortex, extrastriate maps generally have larger receptive field size, stronger levels of normalization, and increased selectivity for second-order contrast. Our results provide insight into how stimuli are encoded and transformed in successive stages of visual processing.

Disclosures: K.N. Kay: None. **J. Winawer:** None. **A. Rokem:** None. **A. Mezer:** None. **B.A. Wandell:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.03/VV7

Topic: D.04. Vision

Support: James S. McDonnell Foundation

NIH-Eureka

National Eye Institute

NIH-Training Grant 5T32NS07467 (SE)

Title: Effects of rest on V4 network coding in perceptual learning

Authors: *S. L. EAGLEMAN, M. MULAS, J. FERNANDEZ-LEON, V. DRAGOI;
Neurobio. and Anat., Univ. of Texas-Houston Med. Sch., HOUSTON, TX

Abstract: It is widely documented that behavioral performance in perceptual tasks is improved following a brief rest. However, the changes in neural network dynamics and coding underlying the behavioral improvement are unclear. In addition, the changes induced by rest in individual neuron and network responses are unknown. We devised a study that had two goals: 1. to determine whether the behavioral performance improvement observed following a period of rest is correlated with an improvement in neuronal discrimination and coding of stimuli, and 2. to employ a battery of analyses during the rest period to identify patterns of neuronal activity during rest (e.g., the presence of replay events and/or pronounced low frequency activity in local field potential responses) that correlate with the improvement in behavioral performance. To accomplish these goals we trained two monkeys to perform a visual same/different task in which successive natural images were judged to have been presented at the same or slightly different orientation. While animals performed the task, we simultaneously recorded the responses of multiple neurons in visual cortex area V4 using custom-made electrode arrays and laminar probes. Each session consisted of a block of trials in which monkeys performed the task followed by a 20-min rest in a dark, quiet room. During this time monkeys were observed to have their eyes closed and jaw-slack (indicative of sleep). Monkeys then performed a second block of trials after rest. We assessed behavioral performance by determining the percentage of correct trials at the monkeys' discrimination thresholds. Following rest, we found a significant increase in the percentage of correct trials. These improvements were correlated with the amount of time animals had their eyes closed during the nap period (Pearson Correlation, r =0.0365, $P < 0.05$). Subsequently, we found improvements in individual neural coding assessed using d' as well as a decrease in the trial by trial correlated variability in firing rate (noise correlation, 20%, Wilcoxon signed rank, $P < 0.001$) which has been previously associated with impoverished stimulus coding. Preliminary results indicate correlative relationships between activity observed during rest and subsequent neural and behavioral improvement. Collectively, our results provide evidence that neural populations exhibit changes in their coding properties that improve network performance following a brief nap.

Disclosures: S.L. Eagleman: None. **M. Mulas:** None. **J. Fernandez-Leon:** None. **V. Dragoi:** None.

Poster

555. Extrastriate Cortex: Neural Coding

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.04/VV8

Topic: D.04. Vision

Support: NIH Grant R00EY020844-03

Klingenstein Fellowship

Whitehall Foundation Grant

NIH Grant 5T32NS7391-14

Title: Attention can adaptively increase or decrease interneuronal correlations in V4

Authors: R. CHANG, D. A. RUFF, *M. R. COHEN;
Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Correlations between the trial-to-trial fluctuations in the responses of pairs of neurons (spike count correlations) can increase, decrease, or have no effect on the amount of information encoded by a neuronal population depending on how information from many neurons is combined. An influential model of perceptual decision-making (Shadlen et al, 1996), predicts that positive correlations among neurons whose responses provide evidence in favor of the same choice are harmful. They reduce the benefit of averaging the responses of many neurons because shared variability cannot be averaged out. In contrast, positive correlations between neurons whose responses provide evidence for opposite choices are helpful because their shared variability can be subtracted out.

Visual attention has long been known to improve perception of an attended location or feature and also to increase the average responses of neurons in visual cortex whose tuning matches the attended location or feature. Recent studies have demonstrated that attention also decreases spike count correlations (Cohen and Maunsell, 2009, 2011; Mitchell et al, 2009; Thiele et al, 2012). In all of these studies, the responses of all of the neurons provide evidence for the same choice, so the correlation decrease could in principle contribute to attention-related improvements in perception. These results are therefore consistent with either of two hypotheses: 1) Attention always decreases correlations or 2) Attention can either increase or decrease correlations, depending on which is more advantageous for encoding sensory information.

To differentiate between these hypotheses, we recorded from groups of neurons in area V4 while monkeys performed a contrast discrimination task. The monkeys viewed two pairs of Gabor stimuli, one pair in each hemifield. In blocks of trials, the monkeys were instructed to indicate which stimulus in the attended hemifield had higher contrast and ignore the other pair of stimuli. We then compared neuronal responses on trials in which the stimuli in the neurons' receptive fields were to be discriminated (and therefore attended) or ignored.

Consistent with previous studies, we found that attention increased average firing rates and decreased correlations among pairs of neurons whose responses contribute to the same perceptual choice. However, we found that attention increased correlations between pairs of neurons that contribute to different choices. Our results show that attention can either increase or decrease correlations and suggest that the effects of attention may depend flexibly on what would benefit the ability of the population to encode sensory information.

Disclosures: R. Chang: None. **M.R. Cohen:** None. **D.A. Ruff:** None.

Poster

555. Extrastriate Cortex: Neural Coding

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.05/VV9

Topic: D.04. Vision

Support: NIH Grant R00EY020844-03

Klingenstein Fellowship

Whitehall Foundation Grant

NIH Grant 5T32NS7391-14

Title: Effects of task difficulty on neuronal populations in visual area V4

Authors: *D. A. RUFF, R. CHANG, M. R. COHEN;
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Abstract: Cognitive factors such as concentration and spatial and feature-based attention are known to affect both performance on psychophysical tasks and also to modulate the responses of individual neurons in visual cortex. Recent studies have made progress understanding the effects of visual attention on measures of population activity. In contrast, our understanding of the effects of global factors like concentration, motivation, or arousal on performance and neuronal populations is much more rudimentary.

Global factors are potentially much easier to manipulate experimentally than other cognitive factors, which makes it useful to know whether they share neuronal mechanisms with processes like attention. If they do, studying whichever cognitive factor is most experimentally accessible would yield general insights. Furthermore, a subject's concentration is likely to fluctuate naturally, which may affect the psychophysical and physiological results of any study of perception or cognition.

We manipulated global cognitive factors by changing the average difficulty of a visual task in blocks of trials. We trained monkeys to indicate which of a pair of Gabor stimuli was of higher contrast. We used three pairs of contrasts, which corresponded to easy, medium, and hard discriminations. We grouped the discriminations in blocks of trials that consisted either of easy and medium discriminations or medium and hard discriminations. Performance on the medium discriminations improved in the context of the difficult blocks, suggesting that the animals

concentrated more when the overall difficulty increased.
Consistent with a previous study (Boudreau et al, 2006), we found that the responses of individual V4 neurons to the medium difficulty stimuli tended to be higher in the hard than the easy blocks of trials. Like spatial attention, increasing the task difficulty also led to a decrease in the correlations between the trial-to-trial fluctuations in the responses of pairs of pairs of neurons. Correlations decreased regardless of whether the stimuli were in the neurons' receptive fields, highlighting the non-spatial nature of this manipulation. We also used the responses of groups of V4 neurons to estimate the animal's concentration on each trial. Fluctuations in concentration affected performance in ways that were predictable but qualitatively different than fluctuations in attention. Our results are consistent with the hypotheses that similar neuronal mechanisms underlie many cognitive factors and that uncontrolled fluctuations in global factors affect the psychophysical and physiological results of many studies, regardless of whether they focus on these factors.

Disclosures: D.A. Ruff: None. **R. Chang:** None. **M.R. Cohen:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.06/VV10

Topic: D.04. Vision

Support: KAKENHI 22135007

Title: Natural texture selectivity of macaque V4 neurons examined by adaptive sampling

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Abstract: Many natural materials are characterized by specific textures that consist of complex combinations of low-level image features such as local contrasts, spatial frequencies and orientations. It has been shown that neurons in ventral higher areas selectively respond to material textures (Köteles et al. 2008: Arcizet et al. 2008), but how the texture selectivity is formed is not known. In this study, in an attempt to understand how the low-level image features are integrated to form selectivity to natural textures, we systematically mapped responses of macaque V4 neurons in a high-dimensional texture parameter space. Texture images were selected from more than 10000 naturalistic textures of 8 materials that were synthesized by an algorithm of Portilla and Simoncelli (2000). Dimension of Portilla-Simoncelli texture synthesis

parameters was reduced by Fisher linear discriminant analysis and the texture images were characterized in 7 dimensional parameter space. To efficiently sample textures that evoke stronger neuronal responses, we introduced an adaptive sampling procedure (Yamane et al. 2008). In the experiment, we first recorded neuronal responses to 50 randomly selected textures, and subsequently we selected other 50 textures such that neighbors of the preferred textures will be more densely sampled. By repeating this procedure, we obtained responses of each neuron to 250 - 500 textures. We were successfully able to fit the observed neuronal responses in the 7 dimensional parameter space and found that the tuning peaks of neurons were widely distributed within the parameter space. Control experiments showed that responses tended to change when the phases of individual Fourier components were randomized but did not change for different textures generated using the same synthesis parameters. The response tunings were largely invariant to the changes in image sizes and positions. These results indicate that neural selectivity to material textures in the ventral higher areas can be understood in terms of the selectivity to higher order image statistics and provide hint on how the low-level image features are combined to form the neural selectivity to natural textures along the ventral visual pathway.

Disclosures: G. Okazawa: None. **S. Tajima:** None. **H. Komatsu:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.07/VV11

Topic: D.04. Vision

Support: NIH Grant NS007391

NIH Grant DA023428

NIH Grant EY018894

NIH Grant EY008098

Research to Prevent Blindness Career Development Award

Research to Prevent Blindness Unrestricted Award

Title: Variance in population firing rate as a measure of slow time-scale correlation

Authors: *M. J. MORAIS[1,2], A. C. SNYDER[1,3], M. A. SMITH[1,3,2];
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Abstract: Correlation among the spiking responses of pairs of neurons, also known as spike count correlation, is a key indicator of functional connectivity and a critical factor in population coding. Importantly, such correlations are not fixed, but are dynamically modulated by the perceptual and cognitive context, underscoring the importance of correlation as a dependent measure for cognitive neuroscience research. Although the context may vary from moment to moment, correlation must by definition be calculated over multiple trials. This property undermines its utility as a dependent measure for investigations of cognitive processes such as selective attention, which have been shown to affect correlation when considered over an entire experimental session, but which presumably fluctuate on a trial-to-trial basis. To investigate the single-trial dynamics of functional connectivity of populations of spiking neurons, it is necessary to identify a measure that can be assayed on a moment-to-moment basis. Here, we introduce the measure of population variance in normalized firing rate for this purpose. We show using simulations and in vivo data how population variance in normalized firing rate is inversely related to the latent correlation in the population, and how this measure can be used to reliably classify trials from different ecologically typical correlation conditions, even when firing rate is held constant. We discuss the potential advantages for using population variance in normalized firing rate as a dependent measure for both basic and applied neuroscience research.

Disclosures: M.J. Morais: None. **A.C. Snyder:** None. **M.A. Smith:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.08/VV12

Topic: D.04. Vision

Support: NIH Grant NS007391

NIH Grant EY018894

NIH Grant EY008098

Research to Prevent Blindness Career Development Award

Research to Prevent Blindness Unrestricted Grant

Eye and Ear Foundation of Pittsburgh, PA

Title: The amplitude and phase of EEG oscillations index the spiking correlation of underlying brain areas

Authors: *A. C. SNYDER[1,2], C. M. WILLIS[1], M. A. SMITH[1,2,3];
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Abstract: An understanding of neuronal communication, both within and between regions of the brain, is fundamental to explaining perception and behavior. Communication among neurons has been studied at a variety of scales, from the relatively coarse resolution afforded by scalp electroencephalography (EEG) to the fine resolution achieved in extracellular microelectrode recordings of pairs of neurons. Measures of functional connectivity such as synchronization of EEG oscillations on a large scale, and correlation of spiking activity among small groups of neurons on a small scale, have each been separately linked to a variety of cognitive and perceptual processes. Although the knowledge gained to date by these parallel efforts has been invaluable, a detailed understanding of neuronal communication will ultimately need to bridge these scales so that the relationships between individual neurons can be understood in the context of the interactions of large-scale brain regions, and vice versa. Here we take the first steps toward this goal with the use of simultaneous EEG and single unit recordings in alert macaque monkeys. Since EEG signals are generally considered to be manifestations of coherent local field potentials (LFPs) generated in cortex, and coherent LFPs would reflect common inputs to that region of cortex, we reasoned that EEG oscillations would be related to the correlation in spiking activity between pairs of neurons in the underlying cortical area. To test this prediction, we recorded population spiking activity from microelectrode arrays chronically implanted in visual area V4 of awake, behaving macaques simultaneously with signals from EEG electrodes on the scalps of the animals. Consistent with our predictions, we found that the amplitude and phase of EEG oscillations indexed the correlation of the underlying spiking activity. Interestingly, we found this relationship between EEG oscillations and spiking correlation to be frequency-specific, which may be related to important functional distinctions between different EEG frequency bands. This is a crucial development that directly links EEG signals to physiological processes with clear computational consequences, and opens an avenue for understanding spiking correlations in terms of the interaction among large-scale brain networks.

Disclosures: A.C. Snyder: None. **M.A. Smith:** None. **C.M. Willis:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.09/VV13

Topic: D.04. Vision

Support: Supported by the Taiwan National Science Council (NSC-96-2811-B-010-501, NSC 97-2321-B010-007), the Taiwan Ministry of Education Aim for the Top University Plan, and Georgetown University development grant GD4235619.

Title: Noise covariations weakly reduce spike ensemble decoding of object content in macaque inferior temporal cortex

Authors: *Y.-P. CHEN[1], C.-P. LIN[1], C. P. HUNG[2];
[1]Inst. of Neurosci. and Brain Res. Ctr., Natl. Yang-Ming Univ., Taipei, Taiwan; [2]Dept. of Neurosci., Georgetown Univ., DC, WA

Abstract: To understand object recognition, it is necessary to characterize the format of the neural code in the inferior temporal (IT) cortex, the last stage of the ventral visual pathway. Are objects encoded by the precise timing of spiking ensembles in inferior temporal cortex, and do slow firing rate covariations (noise covariations) have a strong impact on information coding? Previous studies addressed this issue by examining the entropy of single spike trains, by pooling responses across independent spike trains, or by examining pairwise coincident spiking. However, it remains unclear whether simultaneous measurements of spiking ensembles contains more information than independent recordings of the same neurons. Specifically, we focus on the issue of whether slow trial-to-trial variations are coupled across neurons in an IT ensemble, and the effect of such coupling on object decoding. We recorded simultaneously from local ensembles of ~64 neurons at the lateral surface of macaque anterior IT and compared their synchronous versus asynchronous (trial-shuffled) object category content via a linear support vector machine classifier. The classifier was trained and tested on independent object stimuli, based on spiking responses across a range of ensemble sizes, bin sizes, and analysis windows. As control, we simulated (via spike jittering) the effect of slow covariations in firing rate. Trial-shuffling slightly improved, by less than 10%, category decoding performance across a range of jitter window sizes. These results suggest that simultaneous measurements do not improve decoding based on ensemble firing rates, and that noise covariations in an ensemble only weakly reduce object category content. We speculate that the tuning decorrelation of nearby IT neurons also reduces the impact of noise correlations, and that downstream neurons may further improve decoding by compensating for trial-to-trial firing rate variations in local IT ensembles.

Disclosures: Y. Chen: None. **C. Lin:** None. **C.P. Hung:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.10/VV14

Topic: D.04. Vision

Support: NSF Grant NSF-0640097

NSF Grant NSF-0827427

NEI Grant R01EY017292

Title: Visual selectivity and attentional modulation in V4, IT and the posterior lateral pulvinar

Authors: *E. M. MEYERS[1], R. J. SCHAFER[2], Y. ZHANG[2], T. POGGIO[3], R. DESIMONE[2]; [1]Brain & Cognitive Sci., MIT, CAMBRIDGE, MA; [2]McGovern Inst. for Brain Res., [3]Brain and Cognitive Sciences, McGovern Inst., MIT, Cambridge, MA

Abstract: The neural processing that underlies visual object recognition in primates occurs in the ventral visual pathway, and it is widely believed that this pathway operates in a feed-forward manner where information is passed from one cortical region to the next. It is also known that the pulvinar nucleus of the thalamus is interconnected with visual brain regions, and studies have suggested that all visual cortical regions that have direct anatomical connections also have a connection that passes through the pulvinar. However, it is still unclear what role the pulvinar plays in visual processing, or even how the pulvinar's visual selectivity and attention modulation compares to that of visual cortical areas. To address these questions we recorded neurons in visual cortical areas V4 and the inferior temporal cortex (IT), and from the lateral pulvinar which is interconnected with these regions, while monkeys engaged in a visual attention task. Our results revealed that the population of neurons in the pulvinar was almost as visually selective as populations in V4 and IT, and that the pulvinar contains information in a position invariant format. Additionally, we observed that the magnitude of attention effects in the pulvinar were similar to those seen in V4 and IT. From these findings it appears that the pulvinar inherits its selectivity from the cortical regions it is connected to, and that it might be part of a second pathway that relays visual information between cortical regions.

Disclosures: E.M. Meyers: None. **R.J. Schafer:** None. **Y. Zhang:** None. **T. Poggio:** None. **R. Desimone:** None.

Poster

555. Extrastriate Cortex: Neural Coding

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.11/VV15

Topic: D.04. Vision

Support: EP/F500385/1

BB/F529254/1

Title: Decoding spiking activity in V4, but not V1, correlates with behaviour in perceptual learning

Authors: *S. C. LOWE[1,2], X. CHEN[3], M. VAN ROSSUM[2], S. PANZERI[4], A. THIELE[3]; [2]Sch. of Informatics, [1]Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Inst. of Neurosci., Newcastle Univ., Newcastle, United Kingdom; [4]Inst. of Neurosci. and Psychology, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: When an individual repeatedly performs a simple sensory task, such as discrimination between similar visual stimuli, performance gradually increases, a phenomenon known as perceptual learning. However the neural correlates of this process are not well understood. Here we consider the results of an experiment in perceptual learning of visual contrast discrimination in cortical areas V1 and V4.

In the experiment, a Gabor (V4 recordings) or sinusoidal (V1 recordings) stimulus, with a contrast chosen at random from a set of 14 possibilities, was presented to a macaque monkey. Recordings were made using chronically implanted electrodes in a multi-unit array. The animal was tasked with determining whether the contrast was higher or lower than a control stimulus of 30% contrast; a correct response was met with a water reward. Experimentation continued until performance saturated, after around 20 days.

Using a population-wide linear-discriminant decoding technique based on the mean firing rates during 500ms of stimulus presentation from ~20 channels in V4, we achieved similar levels of performance for the discrimination task, and a similar rate of improvement in performance, as in the monkey's behavioural responses. However, using the same analysis in V1, decoder performance was constant throughout the learning process, despite the animal's improvement in performance. This suggests contrast information present in V1 is invariant throughout learning, whilst V4 improves in its ability to read information from V1.

The trial-to-trial response agreement for the behavioural and decoding responses was statistically significant for V4 (but not V1) when compared with a null hypothesis of independent response generating processes.

By shuffling the neural responses for individual channels across trials with the same stimulus and applying the decoder to this data, the effect of noise correlations on decoder performance was investigated. Although noise correlations do reduce the performance of the decoder, this

reduction does not vary throughout training. Consequently, it is unlikely that perceptual learning for this task is mediated by a reduction in noise correlations in sensory regions.

Disclosures: S.C. Lowe: None. **M. van Rossum:** None. **S. Panzeri:** None. **X. Chen:** None. **A. Thiele:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.12/VV16

Topic: D.04. Vision

Support: NIH Grant EY022538

Title: Neurons in macaque area CIP visually encode the 3D pose of objects

Authors: *A. ROSENBERG, D. E. ANGELAKI;
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Abstract: Interacting with an object in three-dimensional (3D) space often requires the brain to determine the object's 3D spatial pose (i.e., position and orientation) based on visual information. We recently showed that individual neurons in the caudal intraparietal area (CIP) of the macaque monkey visually encode the 3D orientation of a planar surface, and that all orientations are equally represented across the population (Rosenberg, Cowan, & Angelaki; SfN 2012). Because the planar surfaces were always centered on the fixation point in those experiments, it remains unknown whether CIP neurons also encode object position. In the present study, we measure joint tuning curves for the position and orientation of planar surfaces in order to determine if CIP neurons encode 3D object pose.
Position-orientation tuning curves were measured using single-unit extracellular recordings. Stimulus position was varied along left-right, up-down, or forward-backward axes of space, with the central stimulus centered on the fixation point 30 cm in front of the monkey. The translation magnitude ranged between 2.5 and 10 cm. Orientation was varied about either a slant (rotation in depth) or tilt (parallel to the line-of-sight) axis. The planar surfaces were rendered with both checkerboard texture and binocular disparity cues on an LCD screen using red-green anaglyphs. Fixation was maintained within 2 degree version and 1 degree vergence windows. Based on von Mises function fits (average r = 0.93, N=161), orientation preference was largely invariant to position. The average change in orientation preference between fixation centered and displaced object positions was -2±24 degrees standard deviation (N=97 comparisons), and the direction of change was not systematically related to the position (sign test, $p>0.17$). This suggests position

and orientation have multiplicatively separable effects on CIP firing rates, which was tested using singular value decomposition to create a separable model of each position-orientation tuning curve. On average, 86% of the variance in the data (N=63) was accounted for by this model, confirming that position and orientation have largely independent effects on the responses of CIP neurons. Using maximum likelihood decoding, we lastly demonstrate that the position and orientation of a planar surface can be jointly decoded from CIP population activity. Thus, the present results show that CIP can answer two fundamental questions faced whenever it is necessary to interact with an object: Where is it? and How is it oriented?

Disclosures: A. Rosenberg: None. **D.E. Angelaki:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.13/VV17

Topic: D.04. Vision

Support: NIH EY022538

Title: Choice-related activity in area CIP during slant discrimination

Authors: *L. C. ELMORE[1], A. ROSENBERG[1], G. C. DEANGELIS[2], D. E. ANGELAKI[1]; [1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Brain & Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: The ability to construct accurate 3D representations of the world from 2D retinal projections is fundamental to many visually guided behaviors including reaching, grasping, and navigation. A critical step in this process is the precise judgment of the 3D spatial orientation of objects. Recent work in our laboratory has shown that neurons in the caudal intraparietal area (CIP) are tuned for 3D surface orientation. These neurons are jointly tuned for two angles describing 3D surface orientation: tilt (orientation within the frontoparallel plane) and slant (orientation in depth).

To examine the contribution of CIP neurons to slant perception, we recorded extracellular single-unit responses while a rhesus monkey performed a fine slant discrimination task. Stimuli were rendered as random dot stereograms using red-green anaglyphs. Planar slant was defined solely by binocular disparity cues. In a two-alternative forced-choice task, the monkey reported whether a plane was slanted forward or backward by making a saccade to one of two targets. The monkey's mean psychophysical threshold was 3.56°.

We assessed the neural sensitivity of a sample of CIP neurons from one monkey using ROC

analysis. Neurometric functions were plotted for each neuron, and the geometric mean of the neuronal threshold was 26.97±4.57 SD. The average ratio of neurometric to psychometric threshold was roughly 8:1, indicating that single neurons were generally much less sensitive than the animal. We computed choice probabilities to assess the relationship between trial-to-trial variability in neural responses and the animal's choices. The majority of neurons had significant choice probabilities with a mean value (0.58±0.12 SD) that was significantly greater than chance ($p = 0.03$). Also, there was a significant negative correlation between neural threshold and choice probability ($r = -0.48$, $p = 0.05$), such that more sensitive neurons tended to have larger choice probabilities. This relationship between threshold and choice probability is consistent with the hypothesis that perception relies more heavily on the most sensitive neurons. Our findings provide the first evidence of neural activity correlated with the perception of slant, and suggest that area CIP contributes to perception of 3D surface orientation.

Disclosures: L.C. Elmore: None. **A. Rosenberg:** None. **G.C. DeAngelis:** None. **D.E. Angelaki:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.14/VV18

Topic: D.04. Vision

Support: NIH Grant EY11379

NIH Grant P30 EY12196

Title: Joint tuning for direction of motion and binocular disparity may reveal local map structure in MT

Authors: A. SMOLYANSKAYA, D. A. RUFF, *R. T. BORN;
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Abstract: Neurons in sensory cortical areas are tuned to multiple dimensions, or features, of their sensory space. Exactly how single neurons achieve the representation of multiple features is of interest for understanding where specific transformations occur along the visual hierarchy as well as for determining the decoding algorithms appropriate for extracting information from a population of neurons. We investigated the joint tuning of individual MT neurons for two visual features: direction of motion and binocular disparity, an important depth cue. In total we recorded from 92 well-isolated MT neurons in three awake macaque monkeys while they fixed

their gaze on a central point on a computer screen. All combinations of 8 stimulus directions and 7 binocular disparities (-1.2° to +1.2° in 0.4° steps) were presented to two animals, with 15-20 repetitions of each stimulus. The third animal was presented with more finely spaced steps that depended on the preferences of each neuron.

We found that a separable, multiplicative combination of tuning for direction of motion and binocular disparity accounted for more than 95% of the variance in the joint tuning function for over 93% of MT neurons. The separable encoding of these two features indicates that 1) each feature can be read out independently from MT by simply averaging across the population without regard to the other feature and 2) the inseparable representations seen in subsequent areas, such as MST, must be computed beyond MT.

Intriguingly, we found that the remaining non-separable component of the joint tuning function often manifested as small, but *systematic* changes in the neurons' preferences for one feature as the other one was varied. For example, a surprising number of neurons showed a pattern consisting of a counterclockwise rotation of the preferred direction as the binocular disparity was changed from near to far. To determine how frequently such orderly rotations of the preferred direction occurred by chance, we developed a resampling procedure that permuted the disparity information across direction tuning curves while accounting for changes in overall gain that occurred as binocular disparity was varied. This test revealed significant, systematic rotations of the preferred direction as binocular disparity was changed for 34 of the 78 MT neurons for which we had sufficient data to perform the test. We speculate that these non-random rotations reflect the functional organization for direction and binocular disparity in MT and suggest a "like-to-like" local connectivity with respect to both features. If this is correct, joint tuning may provide a new tool with which to probe the local structure of cortical maps.

Disclosures: A. Smolyanskaya: None. **D.A. Ruff:** None. **R.T. Born:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.15/VV19

Topic: D.04. Vision

Support: Supported by the NIMH IRP.

Title: Longitudinal investigation of IT cortex: Delayed emergence of learning-induced plasticity

Authors: *D. A. LEOPOLD[1], A. P. JONES[2], D. B. T. MCMAHON[3];
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Abstract: We recently developed a technique for obtaining longitudinal single unit recordings across days (McMahon et al 2013). As demonstrated by the results presented in the accompanying presentations (Jones et al 2013, Elnaiem et al 2013), visual responses in inferotemporal (IT) cortex are largely stable from day to day in the absence of explicit learning pressure. This observation must be reconciled with evidence that the responses of neurons in IT cortex are shaped by various types of visual experience. For example, norm-based theories of visual function suggest that IT cortex responses are continually recalibrated to reflect the statistical properties of the visual world, at least for specific categories of visual stimuli such as faces. With the goal of understanding how long-term plasticity in IT contributes to acquisition of perceptual expertise, we developed two behavioral paradigms to longitudinally study the effects of learning on single neuron responses in IT. In the first paradigm, monkeys learned to categorize 48 images as "good" or "bad" over the course of less than one hour. In the second paradigm, the animals gradually gained perceptual expertise in discriminating low identity values of morphed human, monkey, and artificial face stimuli. During the course of eight longitudinal experiments conducted in two monkeys in the first paradigm, neurons were tracked from 12 to 37 days. We found that neurons changed their response strength following learning. Changes were not immediate, but generally appeared 1-2 days following the behavioral signs of learning, and were selective for particular stimuli. Changes were sometimes expressed as a scaling up or down of the action potential firing rate and in other cases as an abrupt decrease in response latency. In a few cases, training resulted in neurons gaining new responses to stimuli to which they had previously been unresponsive. The delayed emergence of these experience-induced neuronal changes indicates that visual response plasticity in IT cortex is secondary to learning, but cannot be the causal drivers of learning. We will report these results from the first paradigm along with preliminary data from the second paradigm, which focuses on the acquisition and refinement of perceptual expertise for faces.

Disclosures: D.A. Leopold: None. **A.P. Jones:** None. **D.B.T. McMahon:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.16/VV20

Topic: D.04. Vision

Support: Supported by the NIMH IRP.

Title: Longitudinal investigation of IT cortex: Impact of stimulus repetition across days

Authors: *D. B. MCMAHON[1], I. V. BONDAR[2], D. A. LEOPOLD[3];
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Abstract: Repeated presentations of the same stimuli are commonly associated with improvements in behavioral performance. At the level of single neurons, stimulus repetitions are also associated with reduced spiking responses in monkey inferotemporal (IT) cortex. These findings run contrary to the intuitive expectation that stronger neuronal activity should give rise to faster and better behavioral responses. One hypothesis proposes that the repetition suppression commonly observed over the course of a single recording session contributes to long-lasting decreases in responses to highly familiar stimuli (Li, Miller and Desimone 1993). Since conventional physiological methods typically preclude following activity from the same neurons for more than a few hours, it has not been possible to test this idea experimentally. We recently developed a drivable, MRI-compatible chronic recording technique suitable for longitudinal single unit recording. We first use this technique to demonstrate that the selectivity profile of neurons IT cortex is remarkably stable from day to day, and in some cases across many months. In recordings from two monkeys trained to fixate while the stimuli were presented sequentially, the spiking responses of many neurons (N = 46) decreased strongly over the course of the first seven stimulus presentations (mean reduction = 85%, inter-quartile range = 64%-99%). However, repetition suppression that was observed over the course of a single session did not carry over to the following day. This patten of within-day suppression and across-day recovery to the initial response strength produced a reliable sawtooth pattern in the activity of neurons followed over the course of one month. In the gamma frequency range of the local field potential, the inverse pattern was observed: The amplitude of rectified, band-limited responses in the gamma range increased over repeated trials within a day and returned to low baseline levels overnight. In contrast to the spiking and gamma responses, the broadband evoked field potential did show signs of accumulated changes across days. In particular, the N170 component of the evoked potential decreased in amplitude from 45 microVolts on day one to 23 microVolts on day thirteen. These results show that long-term firing rate reductions as stimuli become more familiar are not an obligatory consequence of repetition suppression observed over shorter, within-session time scales. They instead support the idea that the (presumably deleterious) impact of reduced visual response strength on the fidelity of feature representations might be offset by compensatory changes reflected by the increase in gamma power.

Disclosures: D.B. McMahon: None. **I.V. Bondar:** None. **D.A. Leopold:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.17/VV21

Topic: D.04. Vision

Support: Supported by the NIMH IRP

Title: Longitudinal investigation of IT cortex: Probing category selectivity with 10,000 stimuli

Authors: *A. P. JONES[1,2], D. A. LEOPOLD[2], D. B. T. MCMAHON[2];
[1]Biol., Univ. of Maryland, College Park, MD; [2]Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: In high-level visual areas such as IT cortex, neurons commonly show complex patterns of stimulus selectivity that cannot be readily accounted for by set of simple feature parameters. Response properties of such neurons evidently reflect specialization within a high-dimensional feature space in which the relevant parameters are largely unknown. Our ability to probe the organizing principles of the brain's representational scheme ultimately depends on the number of trials it is possible to obtain for a single neuron. Extending the range of a single experiment to encompass multiple days could therefore shed new light on the principles underlying neuronal specialization. We recently developed a drivable, MRI-compatible chronic recording technique suitable for longitudinal single unit recording. We used this approach to collect responses from neurons in the temporal lobe face patch AF to 10,000 stimuli. Images were drawn from ten object categories that included human faces and body parts, monkey faces and body parts, birds, butterflies, plants, inanimate objects, scenes and Fourier descriptor patterns. Eight trials per stimulus were collected over the course of twelve recording sessions (eighteen days). Consistency in responses to individual stimuli was assessed by a split-halves correlation analysis between odd and even trials ($r = 0.86$). This analysis indicated that the responses obtained were highly reliable despite the fact that they were collected on different sessions spanning more than two weeks. Neurons that gave excitatory responses to human or monkey faces tended to show suppressive responses to whole bodies and body parts, and visa versa. Face cells also responded selectively, albeit more sparsely, to images from non-face categories. Within the face category, AF neurons were selective for identity, face viewpoint, and image scale. Selectivity patterns of nearby neurons (< 1mm apart) tended to carry largely independent signals, as reflected by low correlations across neurons (mean $r = 0.03$, inter-quartile range = -0.04 to 0.11). The independence of neuronal signals in AF was further indicated by principle components analysis, which revealed that the first fourteen principle components only accounted for 85% of the variance across responses. These results demonstrate that longitudinal

recordings have the sensitivity to uncover complex features of neuronal specialization within massive, multi-dimensional feature spaces.

Disclosures: A.P. Jones: None. **D.A. Leopold:** None. **D.B.T. McMahon:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.18/VV22

Topic: D.04. Vision

Support: Supported by the NIMH IRP

Title: Longitudinal investigation of IT cortex: Responses to naturalistic movie stimuli

Authors: *H. D. ELNAIEM, D. B. T. MCMAHON, B. E. RUSS, D. A. LEOPOLD;
Lab. of Neuropsychology, Natl. Inst. of Mental Health, NIH, Bethesda, MD

Abstract: The primate temporal lobe visual system contains specialized regions for processing categories of stimuli that are likely to be of particular importance, such as faces and bodies. Under ecological conditions, the visual world is in constant motion and the dynamic properties of animals and objects are an integral aspect of the sensory environment. In this study, we exploited the ability of chronically implanted microwire electrodes to track cells across days. We used this approach to obtain longitudinal recordings from multiple electrodes while the subjects watched movies showing clips of humans, monkeys, and other animals in naturalistic settings. Chronic electrodes were implanted in the superior temporal sulcus region of inferotemporal (IT) cortex in three monkeys, including one bundle of 64 microwires in face patch AF. Sixty-six neurons from the three animals were strongly modulated by movie content. Individual neurons responded with patterns that were highly consistent across different runs, as well as across days ($r = 0.75$). Correlations between simultaneously recorded neurons located within 1 mm showed weaker, but still significant, correlations ($r = 0.15$). Thus neurons in close proximity (i.e., contained within a single voxel or cortical column) carry largely independent signals. Although neurons outside of face patches responded with a high degree of consistency and selectivity for specific movie content, their responses could not be accounted for by any obvious trigger features. In contrast, the population of neurons in face patch AF responded particularly strongly to scenes showing close-ups of monkey faces. Despite this shared preference for face stimuli, correlations between neurons within AF were no stronger than correlations between neurons from non-face-selective regions. To investigate the underlying dimensions of the neuronal response space, we computed the principle components across a population of 45 simultaneously

recorded spiking responses. The first six principle components together accounted for 85% of the variance across neurons, suggesting a relatively compact dimensionality of feature space. Taken together, these results provide a framework for investigating the specialization of the ventral visual system using a rich, relatively unconstrained regime of visual stimulation.

Disclosures: H.D. Elnaiem: None. **D.B.T. McMahon:** None. **B.E. Russ:** None. **D.A. Leopold:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.19/WW1

Topic: D.04. Vision

Support: NIH Grant R01EY017605

Charles and Johanna Busch Foundation

Title: Transcranial electrical stimulation mitigates motion adaption in V1, MT, and MST neurons of awake, behaving macaques

Authors: *K. KAR, J. DUIJNHOUWER, B. KREKELBERG;
Rutgers Univ., Newark, NJ

Abstract: Transcranial electrical stimulation (tES) is used in the clinic to treat depression, expedite recovery from stroke, and has been used to augment human cognition. Moreover, it is finding increasing use in behavioral neuroscience as a tool to modulate brain activity noninvasively. Its widespread use notwithstanding, the mechanisms by which the externally applied electric fields modulate brain and behavior are still poorly understood. Previously we have reported that tES (10 Hz, 0.5 mA) over hMT+ reduces adaptation in human subjects. This led us to hypothesize that subthreshold rhythmic membrane voltage modulations produced by tES reduce adaptation in motion selective neurons. Here we test this hypothesis with single unit recordings in V1, MT, and MST of the macaque monkey.
We recorded from adapted and unadapted cells with and without tES. The tES electrodes were placed extra-cranially on either side of the recording chamber (over area MT). In the adaptation condition, we presented the adapter stimulus (dots moving coherently in the cell's preferred direction) for 3 s. This was followed by a 300 ms blank period and a 300 ms test phase during which we presented a random dot stimulus moving coherently in one of eight directions. In the stimulation condition the visual adapter stimulus was accompanied by tES (10 Hz, 1.0 mA). In

unadapted control trials, the adapter stimulus was replaced by a noise stimulus consisting of dots moving in random directions.
We measured changes in tuning amplitude and tuning width of the cells and the evoked LFP responses. tES diminished the effects of adaptation on motion selective cells. Notably, whenever adaptation decreased (or increased) tuning amplitude of a neuron, the concurrent application of tES with adaptation reduced this suppression (or facilitation). Similarly when adaptation sharpened (or broadened) the tuning width of a cell, application of tES during adaptation reduced this sharpening (or broadening). Similar mitigation of adaptation was found in the evoked LFP. These results provide novel insight into how tES interacts with neural activity and establishes the awake, behaving macaque as a model to study the mechanisms of tES in-vivo.

Disclosures: K. Kar: None. **J. Duijnhouwer:** None. **B. Krekelberg:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.20/WW2

Topic: D.04. Vision

Support: KAKENHI22135007

KAKENHI24300123

Title: Effects of luminance contrast on the color selective responses in monkey inferior temporal cortex

Authors: *H. KOMATSU[1,2], T. NAMIMA[1,2], M. YASUDA[3], T. BANNO[4];
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Abstract: Luminance contrast of a color stimulus may cause significant influence on the color perception. Although the relationship between color and luminance signals is an important problem in visual perception, relatively little is known about how the luminance contrast affects the responses of color selective neurons.
In the present study, we examined this problem in the monkey inferior temporal (IT) cortex by comparing the responses of color selective neurons at two different luminance contrasts.
Single neuron activities were recorded from the anterior and posterior color selective regions in IT cortex (AITC and PITC) identified in previous studies (Yasuda et al. 2010, Banno et al. 2011)

where color selective neurons are accumulated. Color stimuli (15 bright stimuli and 15 dark stimuli) consisted of 28 stimuli that evenly distribute across the gamut of the CRT display defined on the CIE -xy chromaticity diagram at two different luminance levels (5 cd/m2 or 20 cd/m2) and 2 stimuli at white points. The background was maintained at 10 cd/m2 gray.
We found that the effect of luminance contrast on the color selectivity was markedly different between AITC and PITC. When we examined the correlation between the responses to the bright stimuli and those to the dark stimuli with the same chromaticity coordinates, most AITC neurons exhibited high correlation whereas many PITC neurons showed no correlation or only weak correlation regardless of sharpness of color selectivity. In PITC, the effect was specifically large for neutral colors (white, gray, black) and for colors with low saturation.
These results indicate that the effect of luminance contrast on the color selective responses differs between AITC and PITC. These results suggest that AITC and PITC play different roles in color perception: PITC neurons may play important roles in the distinction between white/black or orange/brown in our color perception, while AITC may do so in the perception of hue across different luminance contrasts.

Disclosures: H. Komatsu: None. **T. Namima:** None. **M. Yasuda:** None. **T. Banno:** None.

Poster

555. **Extrastriate Cortex: Neural Coding**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 555.21/WW3

Topic: D.04. Vision

Support: KAKENHI 22135007

KAKENHI 24300123

Title: Effects of luminance contrast on the color selective responses in monkey visual area V4

Authors: *T. NAMIMA[1,2], G. OKAZAWA[1], H. KOMATSU[1,2];
[1]Natl. Inst. For Physiological Sci., Okazaki, Japan; [2]The Grad. Univ. for Advanced Studies (SOKENDAI), Okazaki, Japan

Abstract: It is well established that there exist color selective neurons in the monkey visual area V4. It has been reported that the responses of many color selective neurons in V4 exhibit selectivity that is invariant to the luminance contrast of the color stimuli (Conway et al, 2009), whereas some neurons are affected by the luminance contrast (Bushnell et al, 2011). However, no attempt has been made to quantitatively compare the responses across the entire range of

gamut including colors with low saturation between stimuli with different luminance contrasts. In the present study, we systematically examined the effect of luminance contrast on the color selectivity of V4 neurons using stimuli that covered the entire range of the gamut.
Color stimuli (15 bright stimuli: 20cd/m^2 and 15 dark stimuli: 5cd/m^2) consisted of 28 stimuli that evenly distributed across the gamut of the CRT display defined on the CIE -xy chromaticity diagram and 2 stimuli at white points. The background was maintained at 10 cd/m^2 gray. Single neuron activities were recorded from the prelunate gyrus in visual area V4 of monkeys performing a visual fixation task.
We found that the effect of luminance contrast varies across V4 neurons and in general responses of V4 neurons were affected by the luminance contrast of color stimuli. When we analyzed the correlation between the responses of the population of color selective V4 neurons to the bright stimulus and those to the dark stimulus having the same chromaticity, we found that the variation in the effect of luminance contrast across colors was large for sharply color selective neurons but small for broadly color selective neurons. In the former, a large effect was stably observed for colors with low saturation.
These results indicate that the luminance contrast significantly affects the neural coding of colors especially for colors with low saturation. The present results are similar to those obtained in PIT cortex in some aspects but different in other aspects (Komatsu, Namima, Yasuda, Banno, this meeting), suggesting that some transformation of color signals takes place between V4 and PIT.

Disclosures: T. Namima: None. **G. Okazawa:** None. **H. Komatsu:** None.**Poster**

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.01/WW4

Topic: D.08. Pain

Support: American Pain Society and the Rita Allen Foundation Award in Pain to MPJ

Title: Sensitization of developing cutaneous nociceptors during peripheral inflammation

Authors: *M. P. JANKOWSKI[1,2], J. L. ROSS[1], J. WEBER[1], A. T. SHANK[1], R. C. HUDGINS[1];
[1]Dept Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Dept. Pediatrics, Univ. of Cincinnati, Cincinnati, OH

Abstract: We have previously found that cutaneous polymodal nociceptors (CPM) are sensitized to heat stimuli in adult mice during inflammation of the hairy skin. We also have found that there is an apparent recruitment of mechanically insensitive, heat sensitive C-fibers (CH). In order to

determine if a similar pattern of sensory neuron sensitization occurred during postnatal development in response to peripheral inflammation, we examined the response properties of cutaneous afferents one day after injection of 3% carrageenan into the hairy hindpaw skin at postnatal days seven and 14. Electrophysiological recordings were performed in male Swiss Webster mice using an *ex vivo* hairy skin-saphenous nerve-DRG-spinal cord preparation. We found that fast conducting sensory neurons had significantly increased firing to both mechanical and heat stimulation of the skin one day after P7 inflammation relative to age-matched naives. We also detected a significant decrease in heat threshold in these fibers at this age. Conversely, one day after P14 inflammation, we did not observe any changes in the fast conducting afferents, but we did detect increased firing to mechanical and heat stimuli in the slowest conducting sensory neurons. In addition, we also observed decreased thresholds to mechanical deformation of the skin in these slowly conducting cutaneous fibers at this age. No changes in fiber distribution were observed at any age at these acute time points. The age-related changes in afferent sensitization correlated with specific upregulation of mechanically and thermally sensitive receptors/channels in the DRGs at distinct ages. For example, while P2Y1, ASIC3, and TRPM3 were upregulated in the DRGs after P7 inflammation, during P14 inflammation, ASIC1, Piezo2 and TRPV1 were uniquely upregulated. These results suggest that sensitization of cutaneous afferents during peripheral inflammation differs depending on the postnatal stage of development. Mechanisms of hypersensitivity to peripheral stimuli may therefore be regulated through different subsets of cutaneous nociceptors and receptors/channels in an age-dependent manner.

Disclosures: M.P. Jankowski: None. **J.L. Ross:** None. **J. Weber:** None. **A.T. Shank:** None. **R.C. Hudgins:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.02/WW5

Topic: D.08. Pain

Support: American Pain Society and the Rita Allen Foundation Award in Pain to MPJ

International Association for the Study of Pain Early Career Grant

Title: Sensitization of group III and IV muscle afferents after ischemia and reperfusion injury

Authors: *J. L. ROSS[1], E. R. COHEN[1], R. C. HUDGINS[1], A. T. SHANK[1], M. P. JANKOWSKI[1,2];

[1]Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Pediatrics, Univ. of Cincinnati, Cincinnati, OH

Abstract: Previous data has shown that inflammation of the muscles alters group III and IV muscle afferent responses to thermal and mechanical stimuli with corresponding changes in gene expression in the affected DRGs. In order to determine if similar changes in response properties and gene expression occur in these afferents during chronic and transient ischemia of the muscles, we surgically occluded the right brachial artery (BAO) of adult male Swiss Webster mice and either maintained the BAO for 24hr or removed the occlusion after 6hr, allowing for 18 hours of reperfusion (I/R). Differences in mechanical, thermal, and chemical sensitivity in muscle afferents were then examined between these two injury states using an *ex vivo* forepaw muscle-median and ulnar nerve-DRG-spinal cord recording preparation. Compared to age-matched naïves, both injury models increased the prevalence of polymodal afferents. Specifically, both chronic BAO and transient BAO with reperfusion induced a significant increase in the number of cells that responded to both non-noxious (low lactic acid and ATP concentrations; pH 7.0) and noxious (high lactic acid and ATP concentrations, pH 6.6) chemical stimulation of the muscles. However, each injury model also induced a unique set of changes in afferent response properties. Chronic BAO induced an increase in firing to heat stimuli in group III and IV muscle afferents that was not detected after I/R. In addition, transient ischemia with reperfusion decreased mechanical threshold and enhanced firing rates to mechanical stimuli, whereas BAO did not differ from the naïve condition in regards to both mechanical threshold and firing. Corresponding with these functional changes in the afferents, each injury state caused upregulation of specific receptors/channels in the DRGs when compared to the naïve condition. Although both conditions showed a significant increase in ASIC3 mRNA, BAO also caused a significant increase in P2X5 and P2Y1, whereas I/R exhibited significant increases in P2X3 and ASIC1, but a significant decrease in P2Y1. Together, these data suggest that ischemic muscle pain may be generated by different mechanisms during chronic ischemia compared to transient ischemia with reperfusion.

Disclosures: J.L. Ross: None. **E.R. Cohen:** None. **R.C. Hudgins:** None. **A.T. Shank:** None. **M.P. Jankowski:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.03/WW6

Topic: D.08. Pain

Support: NS23725 (HRK)

NS050758 (BMD)

NS073548 (KMB)

Title: Firing properties of cutaneous nocieptors in response to natural and optical stimulation in ChR2 transgenic mice

Authors: *K. M. BAUMBAUER, J. J. DEBERRY, B. M. DAVIS, H. R. KOERBER;
Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cutaneous nociceptive fibers are a heterogeneous population comprising many distinct subsets whose individual influence on nociception and/or sensory information processing cannot be easily assessed using naturalistic (e.g. mechanical, thermal, etc.) stimulation. However, with the recent availability of mice modified to express channelrhodopsin, we can now begin to explore the contribution of distinct populations of sensory neurons to nociceptive processing independent of natural transduction mechanisms. The first step in the development of these models is to determine whether light-induced neuronal responses are similar to those evoked by natural stimuli. Transgenic mice expressing channelrhodopsin-2 driven by the Rosa-26 promoter (ChR2-EYFP) were crossed with peripherin-cre or advillin-cre mice. The EYFP tag allowed for visualization of cell bodies and fibers within the DRG and central projections within the spinal cord. When light-induced tail-flick responses were assessed, all mice demonstrated robust responses to as little as a 30 ms flash of light. We next utilized an *ex vivo* skin/nerve/DRG/spinal cord preparation to explore response properties of cutaneous neurons expressing ChR2. Responses were characterized from multiple classes of nociceptors from both strains of mice. Cells were characterized based on their conduction velocities and response to mechanical and thermal stimuli. Once receptive fields were localized, responses to 473 nm laser light transmitted through a 200m diameter optical fiber, were assessed. Nociceptors responded to flashes ranging from 10-5000 ms (39.2 mW/mm^2) and as little as 3.2 mW/mm^2 (1000 ms pulse) of power. Responses to light and naturalistic stimulation were analyzed in mechanically sensitive cells. Suprathreshold light stimulation resulted in tonic firing over the duration of the stimulus while suprathreshold mechanical stimulation evoked a more phasic response. Latency to first response to mechanical and light stimulation were similar. However, peak instantaneous frequencies were significantly higher for suprathreshold mechanical stimulation, averaging 33.9 Hz *vs* 5.4 Hz for optical stimulation (39.2 mW/mm2). These responses were not the result of activation of heat sensitive afferents as the temperature measured at the skin only increased 1°C in response to laser stimulation. These results demonstrate that optical stimulation of the skin in ChR2 mice can activate cutaneous nociceptors, but that optical and naturalistic stimuli are not equivalent. Ongoing experiments are exploring the impact of sensitization on neuronal responses in ChR2 mice.

Disclosures: K.M. Baumbauer: None. **J.J. DeBerry:** None. **B.M. Davis:** None. **H.R. Koerber:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.04/WW7-DP6

Topic: D.08. Pain

Support: NIH Grant NS050758

NIH Grant DK094593

Title: Channelrhodopsin-activation of bladder afferents is sufficient to initiate the visceromotor reflex

Authors: J. J. DEBERRY, H. R. KOERBER, K. M. ALBERS, K. M. BAUMBAUER, *B. M. DAVIS;
Dept. of Med., Univ. of Pittsburgh, PITTSBURGH, PA

Abstract: Sensory innervation of the bladder is comprised of heterogeneous neuronal subpopulations of lightly myelinated and unmyelinated peripheral fibers (A[[Unsupported Character - Symbol Font ]]- and C-fibers, respectively) that convey information about multiple processes, including homeostasis (metaboreceptors) and detection of potentially damaging stimuli (nociceptors). Channelrhodopsin (ChR2) is a light-gated ion channel that allows light activation of neurons. We used Cre-recombinase technology to express ChR2 fused to enhanced-YFP (ChR2-EYFP) in bladder afferents that normally express peripherin or TRPV1 (Per/ChR2; TRPV1/ChR2, respectively). Visualization of the ChR2-EYFP revealed that peripherin-expressing bladder afferents make up the majority of fibers seen in bladder nerve bundles. Fine ChR2-EYFP-positive fibers could be seen leaving the larger nerves and coursing through all bladder layers including the urothelium. In addition, postganglionic neurons in the pelvic ganglion also expressed ChR2-EYFP. TRPV1 promoter-driven ChR2-EYFP fibers were also found throughout the bladder including numerous fine fibers that penetrated the urothelium at regular intervals, perpendicular to the bladder lumen. Using a 473nm laser generating 8-25 mW/mm^2, we were able to induce a foot withdrawal reflex in lightly anesthetized Per/ChR2 mice (1.0% isoflurane). Separate studies (see SfN abstract by Baumbauer et al.) showed that this reflex could be induced with 30ms light pulses, that were only slightly longer than that needed to activate individual nociceptors. This was not a heat-evoked response; thermistor measurements showed that skin temperature did not increase more than 1.0 ^oC upon exposure to the laser. A

laparotomy was then performed to expose the bladder for laser stimulation. A 10 sec stimulation produced a viseromotor reflex that was equivalent to 30-50 mm Hg bladder distention. The reflex onset occurred within the 1.5 sec of laser stimulation (similar to that seen with distension) and continued for the remaining 8.5 seconds. After discharges were also seen occasionally, similar to that observed following bladder distension in the noxious range. These studies show that sufficient ChR2 expression was driven by the peripherin promoter in bladder afferents to evoke bladder reflexes upon light activation. Further, these studies provide proof-of-concept that increases (using ChR2) or decreases (using halorhodopsin) in bladder afferent activity could be effected by selective expression of light-activated molecules and serve as potential treatments for bladder dysfunction.

Disclosures: J.J. DeBerry: None. **H.R. Koerber:** None. **K.M. Albers:** None. **K.M. Baumbauer:** None. **B.M. Davis:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.05/WW8-DP8

Topic: D.08. Pain

Support: NIH Grant NS050758

NIH Grant DK094593

Title: Transgenic expression of endogenous calcium indicator GCaMP3 allows visualization of somatic and visceral sensory neurons *In vivo*

Authors: B. M. DAVIS[1], *J. DEBERRY[1], K. SMITH[2], C. J. WOODBURY[2];
[1]Med., Univ. of Pittsburgh, Pittsburgh, PA; [2]Zoology and Physiol., Univ. of Wyoming, Laramie, WY

Abstract: Both somatic and visceral structures are innervated by numerous types of sensory fibers that are functionally distinct and highly plastic in response to injury and disease. Two central questions in sensory biology are whether disease/injury alter which populations of sensory fibers respond to a given stimulus and the extent to which neurons that were previously silent become functional. To address these questions we crossed mice expressing GCaMP3 (an endogenous calcium indicator) downstream of a loxP-flanked CAG promoter with an EIIa-Cre line in order to induced GCaMP3 expression in all DRG neurons. When dissociated GCaMP3-expressing DRG neurons were examined in culture, low levels of GCaMP3 fluorescence were

detected at rest. When stimulated with 50mM K^+ to induce depolarization and allow Ca^{2+} influx, virtually every cell exhibited a strong Ca^{2+} signal with a αF twice that of Fura-2, but with a longer decay constant ($t_{1/2}$). Stimulation with K^+ could be repeated multiple times (1/min for 20 mins) with no obvious decrease in signal. To determine if GCaMP3 could be used to report activity in somatic and visceral afferents *in vivo*, optical recordings were made in L6 and S1 spinal ganglia in a decorticate preparation. Bipolar electrodes implanted in the base of the tail were driven with stimulus frequencies of 1, 10 and 100 Hz at two different stimulus durations (100 and 500μs). A 100μs pulse was required to elicit a GCaMP3 signal. A GCaMP3 signal was observed at all frequencies with increasing number of cells responding at higher frequencies and the intensity of the GCaMP3 signal increased in individual cells with increased frequency of stimulation. GCaMP3 signals could also be elicited with light brushing, pinch and radiant heat applied to the tail. Light brushing of the tail produced reproducibly strong signals in S1 neurons with large diameter somata. Heating of the tail and perianal region elicited a GCaMP3 signal from neurons with small somata in both L6 and S1 DRG. Both ramp distension (0-60 mmHg over 20 sec) and stepwise square-wave distension (at 20, 40, and 60 mmHg for 20 sec) of the bladder were able to produce GCaMP3 signals. DRG neurons responding to bladder distension exhibited both low (<40 mmHG) and high (>40mmHg) thresholds. Bladder afferents that responded throughout all distension pressures were also observed. These results demonstrate that genetically engineered GCaMP3-expression can be used for population studies of sensory neurons *in vivo*, allowing relatively high throughput analysis of new pain therapies.

Disclosures: B.M. Davis: None. **J. DeBerry:** None. **K. Smith:** None. **C.J. Woodbury:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.06/WW9

Topic: D.08. Pain

Support: NIH Grant NS40538

NIH Grant NS070711

Title: Built to sense: Protein, lipid, and carbohydrate composition of isolated murine dorsal root ganglia neurons

Authors: *M.-E. A. BARABAS[1], E. C. MATTSON[2], C. J. HIRSCHMUGL[2], C. L. STUCKY[1]; [1]Cell biology, Neurobio. & Anat., Med. Col. of Wisonsin, Milwaukee, WI; [2]Physics, UW-Milwaukee, Milwaukee, WI

Abstract: Dorsal root ganglia (DRG) neurons are specialized to detect sensory stimuli such as heat, cold and mechanical force. Sensory neurons are heterogeneous, and specific subpopulations are activated by noxious stimuli, leading to the perception of pain. The identification and characterization of nociceptive subpopulations and their sensory transduction molecules may lead to development of novel pain therapeutics. Chemical components of the neuronal plasma membrane, such as lipids and carbohydrates, may modulate properties of channels and may be used to distinguish subpopulations. In order to study the relevant chemical features of sensory neurons, we conducted infrared (IR) spectromicroscopy on isolated murine DRG neurons. The IR measurements were performed at the Synchrotron Radiation Center (SRC, Madison, WI) with the infrared environmental imaging (IRENI) beamline(1). This approach allows for imaging of chemical functional groups (protein, lipid, carbohydrate) at diffraction-limited resolution on live, unfixed sensory neurons without the use of antibodies or dyes.
We compared IR spectra and chemical images generated from IR absorption of proteins, lipids, and carbohydrates of small-diameter neurons, which are typically nociceptive, with those of large-diameter neurons. The analysis of subcellular localization of chemical features of DRG neurons indicated that some neurons have a lipid- or carbohydrate-dense ring around the perimeter of the somata and the presence of these rings is mainly among large-diameter neurons. It is possible that these rings might provide enhanced sensitivity to large-diameter neurons for activation by innocuous stimuli. We also compared the relative quantities of protein secondary structures throughout isolated neurons and found elevated concentrations of beta-sheet proteins at the soma periphery relative to the interior in both large- and small-diameter neurons. The organization of these protein types may play a vital role in sensory specialization of DRG neurons.
IR spectromicroscopy has not been previously used to study relationships between chemistry, morphology and function in sensory neurons at the single-cell level. Our study demonstrates the potential of this technique to determine relationships between chemical composition and specialized functions of sensory neurons, as well as to potentially identify novel molecular epitopes to define subpopulations of sensory neurons and their transduction moieties.
1.Nasse, M. J.; Walsh, M. J.; Mattson, E. C.; Reininger, R.; Kajdacsy-Balla, A.; Macias, V.; Bhargava, R.; Hirschmugl, C. J. Nat. Methods 2011, 8, (5), 413-U58.

Disclosures: M.A. Barabas: None. **E.C. Mattson:** None. **C.J. Hirschmugl:** None. **C.L. Stucky:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.07/WW10

Topic: D.08. Pain

Support: P01 NS 47399

Title: A subclass of cutaneous polymodal nociceptive C fiber afferents in non human primates responds to β-alanine

Authors: *M. RINGKAMP[1], M. WOOTEN[1], J. BORZAN[2], A. H. KLEIN[1], T. V. HARTKE[1], R. A. MEYER[1];
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Abstract: Two types of heat responses are observed in cutaneous polymodal nociceptive C-fibers when their receptive fields (RF) are exposed to a stepped heat stimulus (49°C, 3s): a quick response (QCs) or a slow response (SCs). We have previously shown that QCs exhibit a greater response to the pruritogen cowhage, whereas the heat response of SCs sensitizes more following a mild burn injury. Others have shown that β-alanine produces itch in humans, and that it activates non-peptidergic, MrgprD positive C fibers in mice. To test if QC and SC afferents differ in their response to β-alanine, we recorded from unmyelinated C fibers innervating the hairy skin in anaesthetized, non human primates using standard teased fiber techniques. After locating the RF, the sensitivity of the afferent fiber to noxious heat was assessed using a contact free, temperature controlled CO2 laser system. A series of 10 µl injections was then administered at the RF: extracellular fluid (ECF), histamine (HIS,10 µg) and β-alanine (ALA, 90 µg). The order of HIS and ALA injections was randomized. Following each injection, neuronal activity was recorded for at least 5 minutes after which recordings were aborted if activity was absent in 3 consecutive minutes. A total of 31 C fibers was tested, 14 of which were classified as QC and the remaining 17 as SC. Responses to ECF were negligible and did not differ between QC and SC afferents. HIS evoked activity did not significantly differ between SC and QC afferents. In contrast, ALA evoked a 4.5-fold larger response in QC than SC afferents. In addition, all QC fibers were activated by β-alanine, but only about one third of SC fibers responded. These results suggest that QC fibers are more sensitive to β-alanine than SC fibers and that QCs may encode the itch associated with β-alanine. The response to a stepped heat stimulus allows identification of β-alanine responsive afferents.

Disclosures: M. Ringkamp: None. **M. Wooten:** None. **J. Borzan:** None. **A.H. Klein:** None. **T.V. Hartke:** None. **R.A. Meyer:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.08/WW11

Topic: D.08. Pain

Support: NIH/NINDS 5-R00-NS-069799

Title: Single-cell labeling of mammalian non-peptidergic nociceptors reveals differences in central projection morphology between axial- and limb-innervating levels

Authors: *W. P. OLSON, J. NIU, A. VYSOCHAN, W. LUO;
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Abstract: Nociceptors are primary pain-sensing neurons located in the dorsal root and trigeminal ganglia. Each nociceptor has one axonal branch elaborating a terminal arbor in the periphery and one central axonal branch innervating the spinal cord. The structure of the peripheral and central arbors of nociceptors defines key aspects of pain sensation (receptive field size, spinal cord connectivity, etc.). Although previous work has revealed the single-cell morphology of some mammalian somatosensory neurons, a systematic characterization of the morphology and development of a defined nociceptor population has not been carried out. To fill this gap, our lab generated a novel inducible Cre mouse line (*MrgD*^{CreERT2}) that allows us to sparsely label non-peptidergic-type nociceptors. When crossed to an alkaline phosphatase reporter mouse line (*Rosa*^{iAP}), AP staining of whole mount skin and spinal cord tissue reveals the morphology of individual peripheral and central terminal arbors of non-peptidergic nociceptors. Interestingly, while the peripheral arbors of this population show a surprising uniformity between body regions, we found clear morphological differences in the central terminal morphology of axial- vs. limb and extremity- innervating neurons. Non-peptidergic nociceptors innervating the trunk have exclusively long and thin central terminals covering ~0.4 segments of the thoracic spinal cord. In contrast, those that innervate limbs, head, and tail display rounded central terminals covering ~0.2 segments of the medial cervical enlargement, medial lumbar enlargement, medulla, and sacral spinal cord. We are currently studying the potential functional relevance of these differences by comparing the postsynaptic partners of these two morphological classes. This work will reveal fascinating novel insights into the neural circuits underlying region-specific functions of the mammalian nociceptors.

Disclosures: W.P. Olson: None. **J. Niu:** None. **A. Vysochan:** None. **W. Luo:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.09/WW12

Topic: D.08. Pain

Support: NIDCD R01 DC011741

NIDCD P30 DC005211

Title: Purinergic modulation of type II cochlear afferents: Sensing trauma in the ear

Authors: *C. LIU[1], E. GLOWATZKI[2], P. A. FUCHS[2];
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Abstract: The mammalian cochlea is innervated by two groups of cochlear afferents - type I afferents form synapses with inner hair cells, and are well known for transmitting acoustic information with high fidelity and efficiency. In contrast, the function of type II afferents has remained mysterious for decades. Despite being postsynaptic to multiple outer hair cells (OHCs), they are found to be 'insensitive' to sound, due at least in part to low probability transmitter release from outer hair cells. In the present study, we asked whether type II afferents also may be driven by purines and pyrimidines released from damaged tissue, as might occur during acoustic trauma. To answer this question, we recorded from type II afferents near their terminal arbors under OHCs in excised cochlear turns from young rats (P7 - P9). 50μM ATP strongly depolarized all tested afferents. ATP-evoked currents reversed at 0mV and could be blocked by PPADS, suggesting the opening of ionotropic P2X receptors. On the other hand, 100μM UTP (a P2Y2 and P2Y4 receptor agonist) also depolarized all the tested afferents, but through a conductance decrease mechanism: the UTP response reversed at -70mV (equilibrium potential for potassium). UTP-evoked current could be antagonized by XE-991, an inhibitor of KCNQ channels, suggesting that UTP activates metabotropic P2Y receptors to close KCNQ channels through second messenger mediated pathways. Although UTP depolarized the type II fibers less potently than did ATP, its closure of KCNQ channels increased membrane resistance, and thus lowered the action potential threshold to enhance excitability. Finally, in order to examine the effect of cell damage, we developed a method that allows us record from type II afferents while OHCs are acutely ablated with a sharp electrode. Strikingly, upon hair cell rupture, a large inward current was recorded from type II afferents, and a slower component of that current was blocked by PPADS, revealing the role of purines as a nociceptive signal. Our results therefore suggest that type II cochlear afferents could sense trauma caused by noxious levels of sound, via their purinergic receptors. The mechanism involves both ATP, the common mediator of tissue damage, and UTP, a sensitizing factor that could lead to hypersensitivity. Our work could have important implications for the pathology of diseases such as tinnitus and hyperacusis, where peripheral damage is thought to lead to central synaptic changes.

Disclosures: C. Liu: None. **E. Glowatzki:** None. **P.A. Fuchs:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.10/WW13

Topic: D.08. Pain

Support: NIDCD R03 DC012413 (M.T.)

NIDCD R01 DC009820 (T.E.F.)

P30 DC04657 (D. Restrepo)

Title: Cholinergic neurotransmission links nasal solitary chemosensory cells to the immune system

Authors: *C. J. SAUNDERS[1,2], M. CHRISTENSEN[1,3], T. E. FINGER[1,2], M. TIZZANO[1]; [1]Dept. of Cell and Developmental Biology, Mail Stop 8108, [2]Neurosci. Program, Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [3]Dept. of Biosci., Aarhus Univ., Aarhus, Denmark

Abstract: The nasal epithelium houses a population of Solitary Chemosensory Cells (SCCs) that respond to “bitter” substances and bacterial metabolites via the canonical taste transduction cascade (T2Rs, Gα-gustducin and TRPM5). SCCs release a hitherto unidentified neurotransmitter onto peptidergic (substance P+) trigeminal nerve endings to evoke protective respiratory reflexes. Until now the possibility that SCCs might also trigger neurogenic inflammation has gone unexplored. In the current study, we developed assays for measuring indicators of inflammation: edema, assayed as plasma leakage, and activation of the innate immune system, assayed as mast cell degranulation (MCD). To measure plasma extravasation (PE), we injected mice i.v. with Alexa555-conjugated albumin, then applied an irritant to the nose. To measure MCD, we stained the respiratory epithelium with acidified toluidine blue, which stains mast cell granules. In both experimental paradigms, mice were stimulated with Denatonium benzoate (Den, 10mM), which stimulates SCC, or with capsaicin (Cap, 2uM), which directly activates trigeminal nocipcetors. Both Den and Cap elevated PE and MCD in normal mice, but only Caps did so in gustducin-/- or TRPM5-/- mice which inactivate SCC transduction. Thus for Den, SCC activation is required for induction of inflammation. Next, we tested mice whose TrpV1-expressing pain fibers had been ablated by resiniferatoxin. In those mice, both Den and Cap fail to evoke PE or MCD, indicating that trigeminal nociceptors are required for these effects. In the lower airways, brush cells, which are similar to SCCs in many respects, utilize acetylcholine (ACh) to signal to nerve fibers. To test whether SCCs too, which like brush cells express choline acetyltransferase, utilize ACh to activate pain fibers, prior to

stimulation, we treated mice with the nicotinic ACh receptor (nAChR) blocker Mecamylamine (Mec). In mice treated with Mec, Den failed to induce PE or MCD, but Caps still evoked normal responses, indicating that nAChR's are required for SCCs to activate trigeminal fibers. Finally, to test the hypothesis that Substance P release from trigeminal fibers triggers PE and MCD regardless of irritant, we treated mice with L732138, an inhibitor of the substance P receptor, neurokinin1. In mice treated with L732138, no significant PE or MCD occured, indicating that activation of NK1 triggers these effects. In summary, we have outlined the pathway by which SCCs trigger neurogenic inflammation in the nasal epithelium first via ACh acting on nAChRs and downstream by release of substance P which acts on NK1 receptors on blood vessels and mast cells.

Disclosures: C.J. Saunders: None. **M. Tizzano:** None. **T.E. Finger:** None. **M. Christensen:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.11/WW14

Topic: D.08. Pain

Support: NSF grant IOS-1146987

NSF grant IOS-1145478

Title: Squid have nociceptors that display widespread long-term sensitization and spontaneous activity after bodily injury

Authors: *R. CROOK[1], R. T. HANLON[2], E. T. WALTERS[1];
[1]Dept. of Integrative Biol. and Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At Houston, Houston, TX; [2]Program in Sensory Physiol. and Behavior, Marine Biol. Lab., Woods Hole, MA

Abstract: Bodily injury in mammals often produces persistent pain that is driven at least in part by long-lasting sensitization and spontaneous activity (SA) in peripheral branches of primary nociceptors near sites of injury. While nociceptors have been described in lower vertebrates and invertebrates, outside of mammals there is limited evidence for peripheral sensitization of primary afferent neurons, and there are no reports of persistent SA being induced in primary afferents by noxious stimulation. Cephalopod molluscs are the most neurally and behaviorally complex invertebrates, with brains rivaling those of some vertebrates in size and complexity. This has fostered the opinion that cephalopods may experience pain, leading some governments

to include cephalopods under animal welfare laws despite minimal evidence for affective states in any invertebrate. It is not known if cephalopods possess nociceptors, or whether their somatic sensory neurons exhibit nociceptive sensitization after noxious stimulation or bodily injury. Previous studies have shown that minor injury to one arm in the squid *Doryteuthis (Loligo) pealeii* promotes a generalized hypervigilant state that persists for days after injury, potentially offsetting the heightened risk of predation that is associated with the presence of injury.
Using extracellular recordings from the fin nerve of the squid, before, during and after a crush injury to the excised fin, we show that squid possess nociceptors that selectively encode noxious mechanical but not heat stimuli, and show long-lasting peripheral sensitization to mechanical stimuli after minor injury to fin tissue. As in mammals, injury in squid can cause persistent SA in peripheral afferents. Unlike in mammals, the afferent sensitization and SA are not restricted to the proximity of the wound site, but are almost as prominent on the contralateral side of the body as they are near an injury. Similar increases in mechanical sensitivity and SA were obtained from squid that had experienced minor injuries inflicted by conspecifics prior to testing.
This distributed sensitization of sensory afferents parallels previous behavioral studies showing increased responses to touch all over the body after a focal injury, and supports previous observations of an absence of directed wound-tending behaviors in squid. Thus, while squid exhibit peripheral alterations in afferent neurons similar to those that drive persistent pain in mammals, robust changes far from sites of injury in squid suggest that persistently enhanced afferent activity provides much less information about the location of an injury in cephalopods than it does in mammals.

Disclosures: R. Crook: None. **E.T. Walters:** None. **R.T. Hanlon:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.12/WW15

Topic: D.08. Pain

Title: Architecture of lumbar spinal cord immediate early gene expression in nociception

Authors: *O. BOJOVIC, D. PANJA, C. R. BRAMHAM, A. TJØLSEN;
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Abstract: The study's objective: Long-term facilitation (LTF) of C-fiber-evoked firing of wide dynamic range neurons in the spinal dorsal horn after conditioning stimulation (CS) of afferent fibers represents a model of spinal nociceptive sensitization and synaptic plasticity. Arc has been

shown to play a role in synaptic plasticity in the hippocampus. Here we wanted to investigate the expression of Arc, C-fos and Egr-1 after a CS which has been shown to elicit LTF.
Methods used: An electrical CS (10 trains of 1 s duration, 10 s intervals, 100 Hz, 1 ms square pulses) delivered to a peripheral nerve has been used in an experimental model of LTF in the spinal cord. Here we examined the effects of this CS of the sciatic nerve in anesthetized female Sprague-Dawley rats on the formation and localization of Arc, C- fos and Egr-1 protein in the lumbar dorsal horn at different time points up to 12 hours post stimulation. The presence of immediate gene proteins after the conditioning stimulation was evaluated by immunohistochemical analysis as well as by western blotting. The detailed location of immediate early gene proteins was examined by fluorescent imaging techniques while cell counting was semi-automatically conducted by FIJI- freeware software.
Results: The numbers of superficially located (laminae I and II) dorsal horn neurons positive for Arc, C- fos and Egr-1 were sharply elevated after the CS compared to the contralateral unstimulated side, and to unstimulated control subjects. Western blotting showed the presence of a band representing increased Arc protein localization at the stimulated side of the spinal cord. The amount of Arc protein seems to be decreasing with time from 1 to 12 hours after CS.
Conclusion: Stimulation of afferent fibers creating LTF increased the number of Arc, C-fos and Egr-1 positive cells in the dorsal horn on the stimulated side of the rat spinal cord. Western blotting showed increased amount of Arc protein on the stimulated side of the spinal cord dorsal horn.

Disclosures: O. Bojovic: None. **D. Panja:** None. **C. R. Bramham:** None. **A. Tjølsen:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.13/WW16

Topic: D.08. Pain

Title: Cutaneous NGF injection in rat: Electrophysiological effects on C-nociceptors revealed by microneurography

Authors: SUMALLA, C. GIAS, R. SOLA, E. GARCIA, M. JONES, J. SERRA;
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Abstract: There is growing evidence that spontaneous impulse generation in hyperexcitable peripheral nociceptors is an important phenomenon in the pathogenesis of neuropathic pain. These abnormally generated ectopic impulses contribute a direct afferent input into the central

nervous system, evoking a sensation of pain, and may set up a state of altered central sensitization that could further amplify the effect of this abnormal input. The intimate mechanisms by which nociceptors may become hyperexcitable are still unclear, but there is growing evidence that NGF may be a key player.
In the present work we have studied the electrophysiological abnormalities induced by NGF in identified subclasses of peripheral C-nociceptors, as a possible surrogate animal model of spontaneous activity in C-nociceptors. We studied the effects of intracutaneous administration of NGF (2 and 5 μg) in the plant of Sprague-Dawley male rats (200-250 g). Microneurographic recordings were obtained at 4 different times: 120 minutes (n=24), 24 hours (n=13), 48 hours (n=15), and 5 days (n=13), in 2 different groups: saline (n=25) vs. NGF (n=40). Activity from individual C-nociceptors was recorded with microneurography, using tungsten microelectrodes inserted into the sciatic nerve trunk.
We recorded from a total of 369 C-nociceptors in the NGF treated group and 302 C-nociceptors in the saline group. Globally, we observed spontaneous activity in 9/302 (3%) of the C-nociceptors of the saline group vs. 74/369 (20.1%) in the NGF treated group. We also observed multispike responses in 5/302 (1%) of the C-nociceptors of the saline group vs. 86/369 (23.3%) in the NGF treated group. The percentage of spontaneous C-nociceptors increased over time from an initial 10.5% at 120 min to 28.9% at 24 h, 22.9% at 48 hours and 22.6% at 5 days. Similarly, there was an increase over time of the frequency of multispike responses from an initial 8% at 120 min to 36% at 24 h, 28.2% at 48 h and 25.8% at 5 days.
In summary, we observed an increased proportion of C-nociceptors displaying spontaneous activity and presence of multispikes due to unidirectional blocks at branching points following NGF treatment in the rat. This experimental paradigm may constitute an excellent surrogate animal model to test the efficacy of new compounds aimed at reducing pathological nociceptor hyperexcitability.

Disclosures: Sumalla: A. Employment/Salary (full or part-time):; NEUROSCIENCE TECHNOLOGIES. **C. Gias:** A. Employment/Salary (full or part-time):; Neuroscience Technologies. **R. Sola:** A. Employment/Salary (full or part-time):; Neuroscience Technologies. **E. Garcia:** A. Employment/Salary (full or part-time):; Neuroscience Technologies. **M. Jones:** A. Employment/Salary (full or part-time):; Neuroscience Technologies. **J. Serra:** A. Employment/Salary (full or part-time):; Neuroscience Technologies.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.14/WW17

Topic: D.08. Pain

Title: Neurotrophic factors regulate scratching behavior and responses of sensory neurons to pruritogens

Authors: *S. DAVIDSON[1], M. V. VALTCHEVA[2], J. P. GOLDEN[2], R. W. GEREAU, IV[2]; [1]Anesthesiol., Washington Univ. Sch. of Med., St Louis, MO; [2]Anesthesiol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Nerve growth factor is increased in the skin of patients with pruritic skin conditions such as atopic dermatitis and is likely involved in concomitant hyperinnervation of the epidermis. Less is known about the potential roles of the Ret activating glial-derived neurotrophic factors in pruritus. We hypothesized that neurotrophic factors modulate scratching behavior and act directly on pruriceptive neurons. This was tested by pretreating mouse cheek skin with a neurotrophic factor (NGF, GDNF, Artemin, or Neurturin) and subsequently recording site-directed scratching behavior evoked by histamine and non-histaminergic pruritogens. NGF increased scratching to histamine, and artemin increased scratching to chloroquine with little increase in cheek wiping, suggesting that neurotrophic factors can increase itch sensation without inducing ongoing pain. The retrograde tracer DiI was injected into the cheeks of mice with eGFP knocked into the Ret locus for identification of Ret+ versus Ret- neurons that innervate the skin. Cultured trigeminal ganglion neurons with identified projection targets to the skin were analyzed in calcium imaging experiments to determine whether neurotrophic factors directly modulate the expression and function of pruritic receptors. Two-thirds of histamine-responsive trigeminal neurons were Ret+. Pre-incubation of cultured neurons with NGF increased the proportion of neurons responsive to histamine. Our results show that neurotrophic factors in the skin can enhance scratching in response to histaminergic and non-histaminergic pruritogens and that neurotrophic factors can act directly on sensory neurons to increase responses to pruritogens.

Disclosures: S. Davidson: None. **M.V. Valtcheva:** None. **J.P. Golden:** None. **R.W. Gereau:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.15/WW18

Topic: D.08. Pain

Support: NIH Grant R01NS48602

Title: Physiological and behavioral responses to pruritogens in the absence of Protein Kinase-Cδ

Authors: *M. V. VALTCHEVA[1], S. DAVIDSON[1], C. ZHAO[1], M. LEITGES[2], R. W. GEREAU, IV[1];
[1]Pain Ctr. and Dept. of Anesthesiol., Washington Univ. Sch. of Med., Saint Louis, MO; [2]Biotech. Ctr. of Oslo, Univ. of Oslo, Oslo, Norway

Abstract: Itch is an unpleasant sensation that elicits the urge to scratch and can present as a debilitating symptom of various dermatological, neurological, and systemic diseases. Itch-producing compounds known as pruritogens stimulate receptors located on C-fibers that innervate the skin and mucous membranes. Most currently known pruritogen receptors are G_q-Protein Coupled Receptors (G_qPCR), which activate the canonical Phospholipase C (PLC) pathway, generating IP_3 and Diacylglycerol (DAG). The specific isoform PLCβ3 is coupled to the histamine H1-receptor, but little is known about the intracellular signaling molecules further downstream and at other pruriceptors. Here, we investigated the role of Protein Kinase-Cδ (PKCδ) as an intracellular mediator of itch signaling in response to various pruritogens. We previously examined PKCδ-knock out (KO) mice in acute tests of mechanical and heat nociception and found no significant change from wild type controls. Immunohistochemistry (IHC) was used to characterize the distribution of PKCδ in mouse dorsal root ganglion (DRG) cells and co-expression with markers for peptidergic and nonpeptidergic neurons. PKCδ expression was restricted to small diameter cells and half of PKCδ-positive cells co-stained for IB4, while a smaller number expressed CGRP. We are currently using *in vivo* and *in vitro* techniques to characterize the effects of genetic deletion and pharmacological inhibition of PKCδ on itch.

Disclosures: M.V. Valtcheva: None. **S. Davidson:** None. **C. Zhao:** None. **M. Leitges:** None. **R.W. Gereau:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.16/WW19

Topic: D.08. Pain

Title: Mutual information analysis of spinal and supraspinal responses to graded electrical stimulations

Authors: *F. G. ARGUISSAIN, J. BIURRUN MANRESA, C. D. MØRCH, O. K. ANDERSEN;
Aalborg Universitet, Aalborg, Denmark

Abstract: Aim: to determine the amount of information carried by single-trial response features extracted from electromyographic (EMG) and electroencephalographic (EEG) signals in response to nociceptive and non-nociceptive electrical stimulation.
Methods: sixteen male subjects participated in a single experimental session, in which they received repeated electrical stimuli to the arch of the foot in order to elicit the nociceptive withdrawal reflex (NWR). Six stimulation intensities (0.5x, 0.75x, 1.0x, 1.25x, 1.5x and 2.0x) multiples of the NWR threshold (i.e. the minimum intensity necessary to elicit the reflex) where applied. NWR features (root-mean-squared amplitude, interval peak z-score, latency and duration) were quantified from EMG signals recorded from the tibialis anterior muscle. EEG features were defined as the amplitudes and latencies extracted from the characteristic peaks (N1, P1, N2 and P2) of the somatosensory-evoked potentials recorded at the vertex. The Information Theory framework was applied to quantify the amount of mutual information (MI) contained in these features with respect to the stimulus. MI is expressed in bits, where zero can be interpreted as a completely random stimulus-response relation and the theoretical maximal value is given by the entropy or self-information of the stimulus (2.58 bits in the present study). Mutual information quantities were calculated on an individual basis and for a subset of combinations of these features.
Results: median MI values of all selected response features from both modalities were informative in regard to the stimulus when considered individually. Significant differences were found among the median MI values of the response features (Kruskall-Wallis test, $P \leq 0.001$). Specifically, the information carried by the EMG features (0.39 bits in average) was significantly higher than information contained in the EEG features (0.11 bits in average) (Dunn's test, p0.05). A preliminary analysis showed that the combination of response features might be more informative than their individual values; however, an overall redundant effect was observed. In most of the combinations, the observed redundancy was small, which might indicate that the stimulus-related information carried by each feature is mainly independent from each other.
Conclusion: these results provide evidence that supports the importance of assessing and interpreting both SEPs and NWR when studying the nociceptive system. The current study presents an alternative approach that allows the quantification of stimulus-signal and signal-signal relationships at single-trial level without linearity and gaussianity constraints.

Disclosures: F.G. Arguissain: None. **J. Biurrun Manresa:** None. **C.D. Mørch:** None. **O.K. Andersen:** None.

Poster

556. **Nociceptors: Anatomical and Physiological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 556.17/WW20

Topic: D.08. Pain

Support: Canadian Institutes of Health Research

Title: μ-Opioid receptor mediated short-term inhibition of Ca2+ signaling and long-term frequency dependent inhibition of action potentials in CGRP nociceptive fibres

Authors: L. D. BAILLIE, *S. J. MULLIGAN;
Physiol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: μ-Opioid receptor agonists are potent analgesics but potential adverse side effects secondary to their central nervous system actions present substantial barriers to their clinical use. Recent studies suggest that peripheral μ-opioid receptors may also be important effectors of systemic opioids in alleviating pain, however, the peripheral cellular mechanisms remain to be established. We have recently introduced an optical microfluorometric imaging approach to directly study physiological functioning within selectively identified, individual nociceptive fibre free nerve endings in a mouse *ex vivo* meningeal preparation (Baillie *et al*. 2011). Calcitonin gene-related peptide (CGRP) pain fibres that originate from the trigeminal ganglion densely innervate the intracranial meninges. We find high density punctate μ-opioid immunoreactive co-localized labeling in the CGRP terminating nociceptive fibres suggesting a peripheral site of action for μ-opioid agonists. Indeed, application of the selective μ-opioid agonist damgo (1 μM) caused a rapid, reversible short-term inhibition in the amplitude of single action potential evoked Ca^{2+} transients that could be blocked with pre-application of the opioid antagonist naloxone (1 μM). After recovery from temporary Ca^{2+} inhibition, we found that μ-opioid receptor activation from localized application of damgo caused long-term failure of action potential propagation. The μ-opioid receptor mediated action potential inhibition is frequency dependent. Short interval action potential firing (for example; 0.25Hz) in control conditions that can be evoked reliably and continuously for extended periods of time, fail in the presence of damgo (1 μM). The short interval action potential firing occurs reliably and continuously with naloxone block of μ-opioid receptor activation. Action potential failures do not occur in the presence of damgo during longer inter-spike intervals (for example; 0.15Hz) demonstrating the frequency dependent inhibition. We find the same effects with the peripherally restricted μ-opioid receptor agonist loperamide. Our research suggests that attacking pain at its source with peripherally restricted opioids may represent a valid therapeutic strategy to achieve potent analgesia while avoiding undesirable central side effects.

Disclosures: L.D. Baillie: None. **S.J. Mulligan:** None.**Poster**

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.01/WW21

Topic: D.08. Pain

Title: Fibromyalgia is a neurological disorder of abnormal central processing mechanisms

Authors: *B. T. SHAHANI[1], R. S. KATZ[2];
[1]Neurol. & Rehabil., UIC Col. Med., Chicago, IL; [2]Rush Univ. Med. Ctr., Chicago, IL

Abstract: Fibromyalgia is usually associated with central pain processing abnormalities, including central sensitization. In addition, we have recently demonstrated that approximately one-third of patients with fibromyalgia syndrome (FMS) also have evidence of peripheral nerve dysfunction, which may contribute to pain, paresthesias, numbness, and fatigue. In this study, we describe three patients, two females (41 and 48 years old) and one male (49 years old) who had no evidence of peripheral neuropathy on detailed electrophysiological studies. Each individual patient described a different sensory perception to the same controlled stimulus, suggesting abnormalities of central processing mechanisms.

Detailed electrophysiological studies including motor sensory nerve conduction as well as late response studies were performed using a TECA EMG machine. Patients were asked to report the sensation they felt when electrical stimulation was delivered to peripheral nerves. The stimulus consisted of single electrical pulses of 0.1to 0.5 msec. duration. The stimulus intensity was adjusted to deliver a supramaximal stimulation.

Motor and sensory nerve conduction as well as late response studies were within normal limits, suggesting that none of these patients had any evidence of peripheral neuropathy.

Supramaximal electrical stimulation of peripheral nerves at the level of the ankles of the lower limbs in the first patient produced a “burning” pain in the feet, which was so intense that she could not control crying during the study.

In the second patient, a single electrical stimulus delivered to the peroneal and tibial nerves produced a ticklish feeling, which elicited uncontrollable laughter on the part of the patient.

In the third patient, electrical studies of median and ulnar nerves produced a dull tapping sensation without any discomfort or dysesthesias.

Electrical stimulation of mixed peripheral nerves produced a distinctly different sensory response in each of these three patients FMS. The stimulus parameter used for this study activated only myelinated fibers; small unmyelinated nerve fibers (C-fibers) were not stimulated. The different types of sensations, including burning and a ticklish sensation experienced by our patients, therefore must have resulted from activation of large and small diameter myelinated nerve fibers. Since the peripheral nervous system was intact in these patients, the perception of burning,

tickling, and the dull tapping feeling must be related to abnormalities of central processing mechanisms.

Disclosures: B.T. Shahani: None. **R.S. Katz:** None.

Poster

557. Psychophysics and Behavior

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.02/WW22

Topic: D.08. Pain

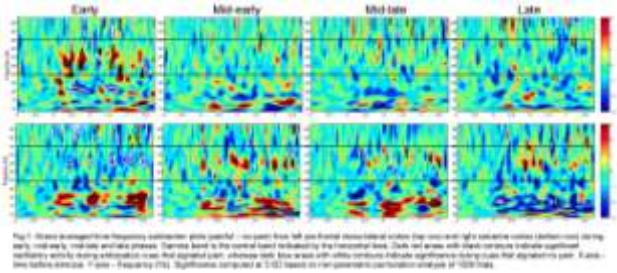
Support: Office of Director, NIH. Grant number: 1DP2OD006469-01

Title: A magnetoencephalography study of early versus late encoding of pain anticipation

Authors: *R. GOPALAKRISHNAN[1], J. C. MOSHER[2], R. C. BURGESS[2], A. G. MACHADO[1];
[1]Ctr. for Neurolog. Restoration, [2]Epilepsy Ctr., Cleveland Clin., Cleveland, OH

Abstract: Central pain syndrome may occur after thalamic strokes or lesions, with contra-lateral numbness or numbness in the body. Not purely a somatosensory experience, chronic pain has significant affective components, including anticipatory phenomena (Melzack 1990). Evaluation of the neural mechanisms underlying pain anticipatory phenomena may be important for understanding pain conditioning and perception. In this study we evaluated for the first time, with magnetoencephalography (MEG), the evolution of electrophysiological activity of underlying neural networks during pain anticipation from the naïve state to the conditioned state. Testing was performed with 5 normal adults seated upright in a 306 channel MEG array (Elekta AB). The paradigm consisted of 4 blocks of 60 randomized trials of distinct visual cues signaling the arrival of a painful stimulus (60% chance) or of no stimulus. Each trial was 9s long with 1s of baseline, 3s of pre-stimulus countdown and 5s post-stimulus period. A contact heat stimulator (Medoc Ltd.) was used to induce pain. The trials were sorted and labeled in four phases as early, mid-early, mid-late and late, the early being naïve and late being conditioned state. The 3s pre-stimulus countdown was pre-processed to the frequency of interest, anomalies rejected and baselined. Minimum-norm source estimation was performed on a tessellated cortex of the brain that was anatomically parcellated. The average time series from each parcel was subjected to a complex Morlet wavelet analysis. The average power estimates were then Z-scored to compute evoked activity. Left pre-frontal cortex showed significant gamma band oscillation to incoming painful stimuli during “early” phase and disappeared thereafter. Interestingly, the visual cortex picked up the gamma activity in the later phases, indicating that the visual cortex gained

encoding independence from frontal areas. The results are indicative of visual cortex initially interacting with higher cortical areas involved in the contextual learning of pain and affective experiences, before becoming independent.



Disclosures: R. Gopalakrishnan: None. **J.C. Mosher:** None. **R.C. Burgess:** None. **A.G. Machado:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Office of Director, NIH. Grant number: 1DP2OD006469-01.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.03/XX1

Topic: D.08. Pain

Support: China NNSF 31171067

China NNSF 61033011

China CAS KSCX2-EW-J-8

China NNSF 31271092

China CAS KSCX2-EW-Q-18

Title: Pre-attentive cognitive processing as indexed by the mismatch negativity (MMN) in tonic experimental pain

Authors: Y. XU, J.-Y. WANG, *F. LUO;
Key Lab. Mental Hlth., Inst. Psychology, Chinese Acad of Sci., Beijing, China

Abstract: Previous studies have shown that pain has modulatory effect on cognitive processes, such as attention. Although the brain mechanisms underlying the interaction between pain and

attention have been explored by numerous cognitive studies, few studies have clarified whether and how pre-attentive processing is influenced in the pain condition. The mismatch negativity (MMN) is an early cognitive component that reflects the automatic detection of a change in a sequence of repeating stimuli, e.g., sounds, and is considered an attention independent process. In the present study, auditory-MMN was recorded in healthy human subjects to investigate the pre-attentive processing under experimentally evoked pain. Cold pressor test (5 ℃ water) was used to produce tonic pain. MMN was elicited using a passive oddball paradigm by frequency changes in sounds consisting of 100-ms tones (standard stimuli: 1000Hz, deviant stimuli: 900/1100Hz). A total of thirty-two healthy subjects participated in the experiment. The MMN was recorded in three conditions (pre-, during, and post-pain), and the amplitude and latency of MMN were compared within subjects. Independent component analysis (ICA) and standard low-resolution tomography algorithms (sLORETA) were employed and combined to analyze the spatiotemporal dynamics of MMN cerebral sources. The results showed that, the MMN which was recorded during pain (when subjects put their hands in the cold water) from the frontal electrode (Fz) was remarkably enhanced compared with the other two conditions. In contrast, positive mismatch potentials of decreased magnitude were detected from the mastoid electrodes (M1 and M2). Moreover, both above measures were correlated with the subjects' pain ratings. ICA and sLORETA analysis revealed that the MMN-related components were located within the temporal and frontal areas which have distinct temporal distribution. Further more, the frontal components were enhanced while the temporal components were reduced during the hand immersion in cold water. These findings raise the possibility that the ongoing pain experience has different influences on the frontal and temporal generators of auditory MMN.

Disclosures: Y. Xu: None. **J. Wang:** None. **F. Luo:** None.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.04/XX2

Topic: D.08. Pain

Support: Heep Fellowship, Texas A&M University

Title: Acute effects of written emotional disclosure on spontaneous pain, neurogenic flare, and secondary hyperalgesia

Authors: *H. R. LINSENBARDT[1], S. K. CREECH[3,4], M. W. MEAGHER[2];
[1]Inst. for Neurosci., [2]Dept. of Psychology, Texas A&M Univ., College Station, TX; [3]Bio Med.

Psychiatry and Human Behavior, Brown Univ., Providence, RI; [4]Providence VA Med. Ctr., Providence, RI

Abstract: The simple act of writing about traumatic experiences (Written Emotional Disclosure, WED) has been shown to result in long-term improvements in health. Individuals with chronic pain, characterized by heightened pain sensitization, have also benefited from WED. Our laboratory recently showed that multiple sessions of WED produce a short-term increase in pain sensitization followed by a long-term decrease in women with trauma history. Although the mechanisms of multi-session WED are beginning to be understood, the acute effects remain understudied. Here we examined the acute effects of WED after a single session of writing on suprathreshold heat (Experiment 1) and on capsaicin-induced spontaneous pain (Experiment 2). Prior studies indicated that WED induces short-term increases in negative affect and arousal leading to our hypothesis of heightened pain following trauma writing. Contrary to our hypothesis, following one session of writing about their most traumatic experience,women reported reduced pain intensity ($F(1,80) = 4.958$, $p < .05$) and unpleasantness ($F(1,69) = 3.7$, $p < .05$) in response to suprathreshold heat as well as reduced ratings of capsaicin-induced spontaneous pain intensity ($F(1, 26) = 4.517$, $p < .05$) and unpleasantness ($F(1, 26) = 4.096$, $p < .05$) compared to participants in a neutral control condition. Data will also be presented on WED's impact on peripheral and central sensitization assessed through neurogenic flare and mechanical secondary hyperalgesia.

Disclosures: H.R. Linsenbardt: None. **S.K. Creech:** None. **M.W. Meagher:** None.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.05/XX3

Topic: D.08. Pain

Support: CIHR Grant 86541

FRQS Doctoral Training Award

CIHR Post-Doctoral Fellowship

Title: The effects of fear conditioning on the psychophysiological responses to pain

Authors: *V. TAYLOR[1,3], M. ROY[4], P. RAINVILLE[1,2,5,3,6];
[1]Psychology, [2]Stomatology, Univ. de Montréal, Montreal, QC, Canada; [3]Ctr. de recherche en

neuropsychologie et cognition (CERNEC), Montreal, QC, Canada; [4]Univ. of Colorado Boulder, Boulder, CO; [5]Ctr. de recherche de l'Institut universitaire de gériatrie de Montréal (CRIUGM), Montreal, QC, Canada; [6]Groupe de recherche sur le système nerveux central (GRSNC), Montreal, QC, Canada

Abstract: Animal studies have shown that a fear conditioned stimulus (CS+) can induce conditioned analgesia mediated by CS-induced amygdala activation sending descending inhibitory signals to the spinal cord. This human psychophysiological study examined whether learning, determined by skin conductance responses (SCR) to the CS+, would alter cortical and spinal responses to a noxious electrical shock (US), assessed respectively with subjective pain ratings and the nociception flexion reflex (NFR). The delay fear conditioning paradigm involved two blocks of acquisition, two blocks of reversal and one block of extinction. In the acquisition phase, two coloured fractal images were presented for 2 sec, one which co-terminated with the shock on 50% of trials (CS+) while the other was never paired with the shock (CS-). In this acquisition phase, 40 CS-, 20 CS+ paired and 20 CS+ unpaired were presented. The reversal phase involved the switching of the CS+ and CS- images. Shocks to the right sural nerve were adjusted individually with an intensity set at 135% of the NFR threshold. NFR was recorded through electromyographic (EMG) recording of the right biceps femoris muscle. Electrodermal activity was recorded on the palmar surface of the left hand. A repeated measures ANOVA revealed a significant PHASE X CS interaction on SCR measures ($F=12.08$, $p=0.0001$). Consistent with the theory of conditioned analgesia, between-subjects correlations revealed significant negative associations between the differential SCR (CS+ vs CS-) and pain ratings to the US in the Acquisition and Reversal blocks (rs between -0.39 and -0.28; ps between .019 and .070). A similar pattern of results was found for the NFR only in the first Acquisition and the second Reversal blocks ($r=-0.31$, $p=0.05$; $r=-0.60$, $p=0.0001$), while the opposite effect was found in the first Reversal block suggesting a facilitation of spinal processes in subjects with faster reversal learning ($r=0.54$, $p=0.0001$). These results are generally consistent with previous studies demonstrating conditioned analgesia but they demonstrate a partial dissociation between spinal and cortical responses suggesting the involvement of at least two separate mechanisms.
Acknowledgements: this work was funded by a CIHR research grant. VT was awarded funding from the FRQS and MR from the CIHR.

Disclosures: V. Taylor: None. **M. Roy:** None. **P. Rainville:** None.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.06/XX4

Topic: D.08. Pain

Support: NIH Grant R01 AR056704

Foundation for Physical Therapy Promotion of Doctoral Studies Award

Title: Associations between chronic stress and pain processing in healthy adults

Authors: B. SHAHIDI[1], M. L. LAUDENSLAGER[2], *K. S. MALUF[1];
[1]Physical Med. & Rehabil., [2]Dept. of Psychiatry, Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Chronic pain disorders have a high comorbidity with stress-related somatic symptoms and have been associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Although acute stress is known to suppress pain sensitivity in both healthy and clinical populations, the effects of chronic stress on pain processing are not well understood. Measurement of hair cortisol has recently been proposed as an index of chronic stress over several months duration. The purpose of this study was to investigate relationships between objective and subjective measures of chronic stress and quantitative assessments of pain sensitivity and central pain processing in healthy individuals. Seventy four healthy adults participated in this study. Hair samples were obtained, and the first 3cm were analyzed for cortisol content as an indicator of chronic stress over the previous 3 months. The Perceived Stress Scale (PSS) was administered as a subjective assessment of chronic stress. Central pain processing was assessed by measuring conditioned pain modulation using a cold pressor test as the conditioning stimulus, and pressure algometry over the upper trapezius (UT) muscle as the test stimulus. This method is thought to elicit endogenous pain inhibitory responses through diffuse noxious inhibitory controls (DNIC). Pressure-pain sensitivity was measured as the threshold for detection of pain during pressure algometry over the UT applied at 1kg*F/sec, and cold-pain sensitivity was measured as the threshold for detection of pain during submersion of the hand in a 38 degree water bath. Hair cortisol levels ranged from 1.07 to 43.73 pg/mg, PSS scores ranged from 3 to 26 points, and DNIC levels ranged from -31.16 to 120.33%. Pressure-pain thresholds ranged from 1.38 to 9.06 kg*F and cold-pain thresholds ranged from 2 to 93 seconds. Results demonstrated no significant associations between hair cortisol and DNIC (r^2=0.0, p=0.88), pressure pain thresholds (r^2=0.0, p=0.99), or cold pain thresholds (r^2=0.02, p=0.27). PSS scores were also found to have no correlation with measures of pain processing($p \geq 0.36$), however, PSS scores were significantly correlated with hair cortisol (r^2=0.11, p=0.004). These results suggest that in healthy individuals, neither objective nor subjective measures of chronic stress are related to pain processing or sensitivity. Further research is needed to investigate the effect of chronic
stress on pain processing in individuals with chronic pain conditions.

Disclosures: B. Shahidi: None. **M.L. Laudenslager:** None. **K.S. Maluf:** None.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.07/XX5

Topic: D.08. Pain

Support: ERC-2010-AdG_20100407

Title: Interaction effects between active treatment and treatment expectation on pain in humans

Authors: *L. A. SCHENK, C. SPRENGER, S. GEUTER, C. BÜCHEL;
Insitute of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Pain relief after administration of an analgesic drug does not merely reflect a pure pharmacological effect - it also depends on contextual factors such as expectation, hope or beliefs about the treatment. The relationship between specific drug and contextual factors is of high theoretical and clinical interest. However, the precise interplay of these factors is currently debated and is still insufficiently understood.
The aim of our study was to investigate possible interaction effects between an active analgesic treatment and treatment expectation at the behavioral and neuronal level. We combined topical lidocaine-prilocaine cream with an expectancy manipulation in a 2x2 within-subject design (open treatment, hidden treatment, placebo, control). 32 healthy subjects received heat pain stimuli on capsaicin pretreated skin and rated their experienced pain during a functional magnetic resonance imaging experiment. Conditions were counterbalanced across subjects. Our design allowed us to separate drug-related and expectancy-related effects at the behavioral and neuronal level and to test whether they interact during the processing of painful stimuli.
Pain ratings revealed a significant main effect of active treatment and a significant interaction between active treatment and treatment expectation. The expectation effect was significantly larger in the treatment conditions (open treatment versus hidden treatment) compared to the no-treatment conditions (placebo versus control). Active treatment was associated with reduced activity in the anterior insular cortex. The interaction was related to signal changes in several placebo-associated brain areas, including the anterior insular cortex, the anterior cingulate cortex and the ventral striatum.
In conclusion, this study shows that synergistic effects between active treatment and treatment expectation are found in behavioral pain ratings and are associated with responses in placebo-related brain regions. Our results are relevant for clinical studies where additive effects of drug and context are presumed, as well as for clinical practice, which could benefit from a better understanding of synergistic effects between therapy and context.

Disclosures: L.A. Schenk: None. **C. Sprenger:** None. **S. Geuter:** None. **C. Büchel:** None.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.08/XX6

Topic: D.08. Pain

Support: BMBF grant 01EC1010D

Title: Deficient modulation of pain by a positive emotional context in fibromyalgia patients

Authors: *H. FLOR, I. C. BOMBA, E. DIESCH, S. KAMPING;
Dept. of Cognitive and Clin. Neuroscience, CIMH, Heidelberg Univ., Mannheim, Germany

Abstract:
Fibromyalgia syndrome (FMS) is marked by chronic widespread pain and is accompanied by additional symptoms ranging from fatigue, stiffness, sleep disturbances, to depressive symptoms. Lang's motivational priming hypothesis (Lang PJ, Bradley MM, Cuthbert BN. (1990) *Psychol Rev.* 97:377-398) suggests that an organism's motivational state will modify its responses to stimuli, with enhanced pain perception to negative and decreased pain perception to positive picture stimuli. We aimed to investigate the modulating effects of emotional context on pain perception in 16 patients with FMS and 16 healthy control (HC) subjects. An infrared laser was used to apply individually adapted painful stimuli to the dorsum of the left hand. The emotional background of the painful stimuli was modulated by concurrent presentations of negative, neutral, and positive picture stimuli selected from the International Affective Picture System. As control conditions, painful stimuli and the pictures were also presented by themselves. During each of the five laser-picture trials, subjects received 10 painful stimuli and were asked to rate the average intensity and unpleasantness of the experienced pain. Functional magnetic resonance images were obtained, using a T2* sensitive echo planar sequence. HC subjects showed a linear increase in pain intensity and unpleasantness ratings when painful stimuli where presented during positive, neutral, and negative pictures. In contrast, FMS patients showed a quadratic trend for pain intensity ratings indicating a lack of pain reduction by the positive pictures. In addition, the FMS patients showed less activation in secondary somatosensory cortex, insula, orbitofrontal cortex, and anterior cingulate cortex during the positive picture pain trials. Our results suggest that fibromyalgia patients are less efficient in modulating pain by positive affect and may benefit less from appetitive events than healthy controls. In light of our results we propose that therapeutic approaches for the treatment of FMS should place a stronger emphasis

on emotional processes and try to normalize the disrupted affective modulation.
Support: This work was supported by the German Ministry of Education and Research as part of the research consortium LOGIN (01EC1010D).

Disclosures: H. Flor: None. **I.C. Bomba:** None. **E. Diesch:** None. **S. Kamping:** None.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.09/XX7

Topic: D.08. Pain

Support: Idea2

Gollub/Kaptchuk R01

Title: Effects of a personal music-enhanced conditioning paradigm on heat pain analgesia

Authors: C. HSIEH[1], J. KONG[2], *R. L. GOLLUB[2];
[1]Hlth. Sci. and Technol., MIT, Cambridge, MA; [2]Dept. of Psychiatric Neuroimaging, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Introduction
The experience of pain has both sensory and affective elements, and can be modulated with cognitive distractions as well as emotional manipulation. Placebo analgesia studies have shown an effect from verbal expectancy manipulation, which can be additionally boosted through covert conditioning. More recently, there have been a number of demonstrations of music's analgesic capabilities in experimental settings. Music has been shown to both reduce anxiety and activate reward circuitry; because both factors are part of the neural pathways modulating pain perception, it is conceivable that music could access one or both pathways as an analgesic vehicle.
Materials and Methods
A two-session experiment was performed with 48 healthy, pain experiment-naïve subjects (age 27.1 ± 7.1, 15 males). The first 36 were randomized into one of 3 treatment groups, including music conditioning, sound conditioning, and no-treatment routine pain calibration. A second music conditioning group of 12 subjects was completed after the first cohort. Primary outcome measures included the difference (pre- minus post-treatment) in subjective pain rating to calibrated experimental noxious heat stimuli. We also collected treatment expectations using an Expectancy of Relief Scale (ERS).

Results

Expectations for the sound sample remained significantly lower throughout the study in the calibration group (Fig. 1a), but were increased in the sound conditioning group by verbal suggestion. Music expectations were not increased above calibration level by verbal suggestion but were increased by conditioning, however conditioning did not further increase analgesia induced by music (Fig. 1b). Conditioned sound did not reduce pain intensity, but sound reduced pain unpleasantness.

Conclusion

The results here suggest a non-additive interaction between personal music's analgesic capacity and that from expectancy-based conditioning. Strongly personal stimuli may invoke a ceiling effect rendering conditioning ineffective at enhancing analgesia.

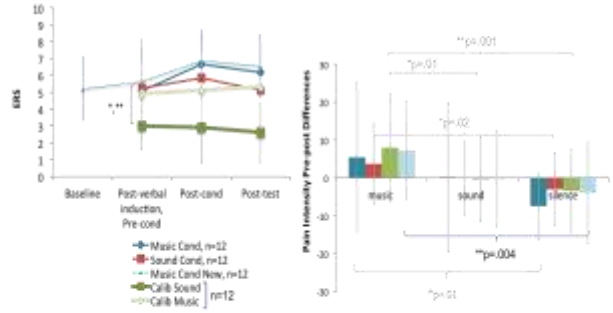


Fig. 1a: Expectancy differed significantly within the calibration group between expectations for music and that for the sound sample (**p<.001). This difference was seen at all time points the ERS was assessed, with a slightly less significant difference after testing. The verbal induction boosts expectations significantly higher (*p=.03) in the sound conditioning group, to the level of music expectations. In the new music conditioning group, an additional assessment of ERS at a completely naive baseline in this group shows that verbal induction did not significantly raise expectations for music. We also see a replication of pre-conditioning ERS scores for music conditioning.

Fig. 1b: Within the calibration group (green bars), music reduced pain intensity significantly more than sound and silence. In the sound conditioning group (red bars), only unconditioned music reduced pain, as compared to silence. In the original music conditioning group (turquoise), only music reduced pain as compared to silence, with neither magnitude or significance greater than that seen in the calibration group. In the second music conditioning group (light turquoise), music again reduced pain as compared to silence, with a slight increase in significance compared to the original music conditioning group.

Disclosures: C. Hsieh: None. **J. Kong:** None. **R.L. Gollub:** None.

Poster

557. Psychophysics and Behavior

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.10/XX8

Topic: D.08. Pain

Support: King Research Excellence Award

Title: Cool adaptation reduces the pain of the thermal grill illusion

Authors: *D. E. HARPER, M. HOLLINS;
Psychology, Univ. of North Carolina, Chapel Hill, NC

Abstract: The thermal grill illusion (TGI), a phenomenon in which interlaced warm and cool bars produce a painful sensation, is not completely understood. The present study used thermal adaptation as a tool to elucidate the relative contributions of warm and cool signals to the illusion. Each subject (n=24; 14 female) underwent three experimental runs, in counterbalanced order. Two runs tested the effects of pre-adapting the subject to either cool bars (18°C) or warm bars (42°C), interlaced with neutral bars (32°C), for a period of 3min. Following pre-adaptation, the neutral bars were warmed or cooled to produce a canonical thermal grill (i.e. 42°C bars interlaced with 18°C bars). Both sets of bars were held at the neutral temperature during the pre-adaptation phase of the remaining run, to measure baseline sensitivity to the TGI. Subjects rated thermal sensations and pain at various times during each run using computerized visual analogue scales (VAS). Thermal ratings during pre-adaptation revealed significant thermal adaptation, with warm/cool sensations gradually weakening over the course of 3 min. There were no differences in the perceived intensity of these warm and cool sensations, or in the amounts by which they declined. A repeated-measures ANOVA showed a significant difference in the painfulness of the TGI across runs. Compared to the pain of the TGI during the baseline run (M=29.9), cool adaptation significantly reduced pain (M=13.6) while warm adaptation was without effect (M=26.4). This result indicates that the grill's cool bars have a selective role in supplying the pain of the TGI. Most subjects perceived the TGI in the baseline condition to be hotter than the grill's warm bars alone; following warm adaptation, however, a majority instead rated the TGI as cool or cold. The subjects who found the TGI to be cool or cold following warm adaptation also found it to be significantly less painful, following both warm and neutral pre-adaptation, than did those who perceived it as hot. In fact, the more often a subject reported feeling cool or cold from the TGI across all runs, the less pain he or she reported from the TGI. Several studies have shown that there is a great degree of variability in susceptibility to the painfulness of the TGI and some researchers have even questioned whether the TGI is actually painful. The present study provides a possible explanation for this variability - cool's susceptibility to be masked by warmth (a mechanism of the TGI proposed by Craig and Bushnell, 1994) might differ across individuals, shielding some from the painful burn of the TGI.

Disclosures: D.E. Harper: None. **M. Hollins:** None.

Poster

557. **Psychophysics and Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 557.11/XX9

Topic: D.08. Pain

Support: R01DE18214

R01DE18538

P20DA26002

Title: Brain indoleamine 2,3-dioxygenas regulates nociceptive and depresion- like behavior in genetically predisposed depression-like behavior rats

Authors: *H. KIM[1], M. MCCABE[2], G. LIM[3], L. CHEN[3], J. MAO[3];
[1]Anesthesia, MGH, CHARLESTOWN, MA; [2]MGH, boston, MA; [3]MGH, Boston, MA

Abstract: We demonstrasted that persistant nociception induces both depression-like behavior and upregulation of brain indoleamine 2, 3-dioxygenase (IDO), a rate-limiting enzyme in tryptophan metabolism. Here, we demonstrate that brain indoleamine 2,3 dioxygenase 1 (IDO1) expression was elevated in genetically predisposed depression-like behavior rats (WKY rats). As compared with Wistar rats at the basal level, WKY rats showed 1) an increased immobility time in FST, 2) an increased hippocampal IDO expression and 4) an increased kynurenine/tryptophan ratio but a decreased serotonin/tryptophan ratio in the hippocampus. Moreover, WKY rats exhibited a lower baseline nociceptive threshold in both mechanical and thermal tests as compared with Wistar rats.
Predisposed depressive behavior in WKY rats exacerbated mechanical allodynia, thermal hyperalgesia, and depressive behavior following the CFA injection into the right tibio-tarsal joint as compared with sham controls. Moreover, the hippocampal IDO expression was further upregulated in WKY rats with arthritis which resulted in a further elevated kynurenine/tryptophan ratio and a further reduced serotonin/tryptophan ratio in the hippocampus of these WKY rats. These results, together with the data from Wistar rats, demonstrate a reciprocal relationship between pain and depression that is linked through IDO expression and activity in the hippocampus.
Keyword: Pain; Depression; Indoleamine 2,3-dioxygenase; Tryptophan; Kynurenine; Serotonin

Disclosures: H. Kim: None. **M. McCabe:** None. **G. Lim:** None. **L. Chen:** None. **J. Mao:** None.**Poster**

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.01/XX10

Topic: D.08. Pain

Support: NSF 1051734

Title: Cannabinoids differentially modulate behavioral responses to nociceptive vs non-nociceptive stimuli

Authors: *T. L. RASMUSSEN, B. D. BURRELL;
Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: The endocannabinoid system is thought to play a role in modulating nociception, making it a potential therapeutic target for treating pain. The anti-nociceptive effects of endocannabinoids appear to be due, at least in part, to a depression of excitatory synaptic transmission within pain neural circuits. However, conflicting results from both clinical and laboratory-based studies have found that endocannabinoids can also pro-nociceptive effects, contributing to pain-related sensitization (e.g. mechanical hyperalgesia or allodynia) due to disinhibition of synapses from primary afferents. Using the medicinal leech as a model system, our lab has observed that while endocannabinoids differentially modulate somatosensory signaling; depressing nociceptive afferent synapses, but potentiating non-nociceptive afferent synapses (Yuan & Burrell *J. Neurophysiol.* 104: 2766, 2010; Higgins et al. *Molec. Pain* under review). In the present study, we examined cannabinoid treatment had a similar differential effect on behavioral responses to nociceptive vs. non-nociceptive stimuli. Leeches were injected with either the synthetic cannabinoid CP 55940 (0.11 mg/g) or the endocannabinoid 2-arachidonoylglycerol (2-AG; 0.28mg/g). Responses to non-nociceptive stimuli were monitored by measuring the threshold for eliciting sucker withdrawal in response to von Fray fiber stimulation. Responses to nociceptive stimuli were tested by applying capsaicin to the posterior sucker and observing latency to move away from the treatment area and duration of this nocifensive locomotion. Both CP 55940 and 2-AG significantly sensitized the animals to non-nociceptive stimuli, indicated by a decrease in the response threshold to von Frey fiber stimulation. Simultaneously, 2-AG and CP 55940 decreased responses to nociceptive stimuli, i.e. increased latency to move after capsaicin application and decreased the duration of nocifensive locomotion. Additional studies are currently underway to examine the duration of these effects and if both forms of cannabinoid-elicited modulation are mediated by central Transient Receptor Potential (TRP) channels. These findings provided behavioral support for the hypothesis that the pro- and anti-nociceptive effects of cannabinoids are due to differentiation modulation of nociceptive vs. non-nociceptive signaling.

Disclosures: T.L. Rasmussen: None. **B.D. Burrell:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.02/XX11

Topic: D.08. Pain

Support: NSF-1051734

Title: Differential effects of GABA in modulating nociceptive vs. non-nociceptive synapses

Authors: Y. WANG, B. D. BURRELL;
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Abstract: Treatment of pain is a major health concern in the United States and there is considerable interest in developing new analgesic therapies. Cannabinoid-based treatments represent one potential approach, but results from both laboratory and clinical studies have observed both pro- and anti-nociceptive effects of cannabinoids. How can the cannabinoids have these opposing effects? Using the leech as a model system, our lab has observed that endocannabinoids can depress synapses from nociceptive neurons, but increase synaptic transmission by non-nociceptive neurons, potentially recruiting these cells into the nociceptive circuitry (Yuan & Burrell J. Neurophysiol. 104: 2766, 2010; Higgins et al. Molec Pain under review). These increases in synaptic signaling appear to be due to endocannabinoids causing a decrease in inhibitory GABAergic signaling (disinhibition). However, this leads to the question of why nociceptive synapses are not potentiated/ disinhibited by endocannabinoids as well. These opposing effects may be due to differences in effects of normally inhibitory neurotransmitter GABA.

We have observed that GABA can hyperpolarize/inhibit non-nociceptive sensory neurons, but depolarize/excite nociceptive neurons (Higgins et al. Molec Pain under review). GABA's effects are dependent on the levels of intracellular Cl-, which is controlled by two Cl- transporters: Na-K-Cl co-transporter isoform 1 (NKCC1), which imports Cl- and K-Cl co-transporter isoform 2 (KCC2), which exports Cl-. GABA-induced depolarization of the nociceptive afferent was reduced by the NKCC1 inhibitor bumetanide. The GABA-A receptor antagonist, bicuculline, depressed N-cell synaptic transmission, indicating that GABA-mediated depolarization was in fact excitatory. Bumetanide depressed N-cell synaptic transmission as well, consistent with the hypothesis that the excitatory effects of GABA on nociceptive synaptic signaling are the result of a depolarized Cl- equilibrium potential. GABA-induced hyperpolarization of the non-nociceptive afferent was reduced by the KCC2 inhibitor VU 0240551, which also caused a depolarizing shift in the chloride equilibrium potential. Bicuculline disinhibited non-nociceptive synapses and experiments are currently underway to determine if VU 0240551 can also affect these synapses. These findings suggest that the "sign" of GABAergic signaling plays a critical role in regulating the modulatory effects of endocannabinoids at the synaptic circuit level.

Disclosures: Y. Wang: None. **B.D. Burrell:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.03/XX12

Topic: D.08. Pain

Support: NMRC/1255/2010

Title: Medial septum GABAergic mechanisms preferentially modulate formalin-induced moment-to-moment agitation and aversion

Authors: *S.-T. ANG[1,2], A. T. H. LEE[1,2], Z. M. ARIFFIN[1,2], L. NG[1,2], A. VIPIN[1,2], C.-M. LOW[1,3,4], S. KHANNA[1,2];
[2]Physiol., [3]Pharmacol., [4]Anaesthesia, [1]Natl. Univ. of Singapore, Singapore, Singapore

Abstract: The forebrain medial septum (MS) is implicated in nociception. Thus, hind paw injection of formalin in rat, a model of tonic inflammatory pain, excites septohippocampal neurons and lead to the generation of theta rhythm in the hippocampus which is also implicated in formalin nociception. Conversely, inactivation of the MS with the GABAA receptor agonist, muscimol, attenuated formalin-induced nociceptive licking and flinching and c-Fos induction in the spinal cord. Interestingly, the MS GABAergic neurons are implicated in the generation of the hippocampal theta rhythm that indirectly suggests that these are excited by noxious stimulation. Thus, the objective of the present study was to examine the role of MS GABAergic mechanisms in a spectrum of pain-induced behaviors, including (i) formalin-induced acute nociceptive behaviors and agitation, (ii) pain-induced associative learning in a contextual fear-conditioning task, (iii) pain-induced avoidance learning using the formalin-induced conditioned place avoidance (F-CPA) task, and (iv) the expression of peripheral hypersensitivity (PH) in the complete Freund's adjuvant model (CFA) of chronic inflammatory pain. The role of MS GABA in formalin-induced acute behaviors was assessed directly by microinjection of bicuculline and by selectively destroying the GABA neurons with the neurotoxin, kainic acid. The effect of GABAergic manipulations was compared with intraseptal microinjection of the NMDA receptor antagonist, AP5, in doses that attenuate septohippocampal theta activation. Subsequently, the influence of destruction of MS GABAergic neurons was assessed on fear-conditioning, F-CPA and CFA-induced PH.
In awake animals, intraseptal microinjection of bicuculline, in dose that attenuated theta activation in anaesthetized rat, and AP5 had no effect on formalin-induced nociceptive licking and flinching. However, these drugs enhanced the formalin-evoked agitation. Likewise destruction of GABAergic neurons (more than 70%) by kainic acid did not affect formalin-

induced nociceptive behaviors and PH in the CFA model, though a trend towards increased agitation was observed in the formalin test. The destruction of GABAergic neurons, however, attenuated freezing response in contextual fear conditioning and abolished avoidance behavior to the pain-paired compartment in the F-CPA task. Taken together, the study provides evidence that MS GABAergic mechanisms modulate pain-induced affect and sensorimotor integration. Moreover, the evidence suggests that MS mechanisms that modulate pain affect are functionally separate from those that underpin pain-induced acute nociceptive behaviors.

Disclosures: S. Ang: None. **A.T.H. Lee:** None. **Z.M. Ariffin:** None. **L. Ng:** None. **A. Vipin:** None. **C. Low:** None. **S. Khanna:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.04/YY1

Topic: D.08. Pain

Title: Increase in spontaneous saphenous nerve activity after oxaliplatin administration: a possible index of spontaneous dysesthesia

Authors: *T. ANDOH, S. MIYAO, P. GAUCHAN, Y. KURAISHI;
Univ. Toyama, Toyama, Japan

Abstract: Oxaliplatin causes peripheral neuropathy, a dose-limiting side effect of anti-cancer drugs. Oxaliplatin-induced peripheral neuropathy is characterized by pain and dysesthesias, such as spontaneous electrification-like sensation and cold dysesthesia, in the hands and feet. Cold dysesthesia and mechanical allodynia have been studied in rodents, because they can be behaviorally assessed. However, spontaneous pain and dysesthesia are difficult to behaviorally examine in rodents. In this study, therefore, we examined whether spontaneous pain and dysesthesia associated with oxaliplatin-induced peripheral neuropathy can be electrophysiologically assessed in mice. Male C57BL/6 mice were used. A single intraperitoneal injection of oxaliplatin (3mg/kg) or vehicle (5% glucose) was administered. Recordings were made from the saphenous nerve that innervates the medial-dorsal hindpaw. Mechanical allodynia and cold dysesthesia were evaluated using a von Frey filament (0.69 mN) and acetone, respectively. Spontaneous saphenous nerve activity significantly increased on days 3, 10, and 32 after oxaliplatin injection, peaking on day 10. Licking, a pain-related behavior, of the hindpaws did not increase on day 3, 10, and 32. Mechanical allodynia and cold dysesthesia peaked on day 10 and day 3 after oxaliplatin injection, respectively, and almost subsided on day 32. The

response of the saphenous nerve to von Frey filament stimulation increased on days 3, 10, and 32 after oxaliplatin injection, peaking on day 10. Saphenous nerve response to acetone stimulation increased on days 3 and 10 after oxaliplatin injection, peaking on day 3. We found for the first time that spontaneous saphenous nerve activity increased after oxaliplatin administration. Although further studies are needed, the present results raise the possibility that increased spontaneous activity of the saphenous nerve is associated with oxaliplatin-induced spontaneous dysesthesia, including electrification-like sensation.

Disclosures: T. Andoh: None. **S. Miyao:** None. **P. Gauchan:** None. **Y. Kuraishi:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.05/YY2

Topic: D.08. Pain

Support: NIH R01 NS080954-01

Stanford Bio-X Neuroventures Program

Stanford Bio-X Interdisciplinary Initiatives Program

Title: Bidirectional virally-mediated optogenetic control of pain

Authors: *S. M. IYER, K. L. MONTGOMERY, C. L. TOWNE, S. Y. LEE, C. RAMAKRISHNAN, K. DEISSEROTH, S. L. DELP;
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Abstract: Optogenetic control of primary nociceptors could potentially have great translational and basic research value. Here, we demonstrate bidirectional optogenetic control over pain perception in non-transgenic, virally injected, freely moving mice, through non-invasive illumination of the mouse environment. We use injection of wild-type mice with adeno-associated virus to express opsins in unmyelinated nociceptors. We show that blue light illumination of the feet of mice injected with AAV6-ChR2 (channelrhodopsin-2) is aversive, and appears to result in characteristic pain-like behavior. Interestingly, we also find that low intensities of blue light that are insufficient to induce outward signs of pain can still induce place aversion, and sensitize mice to mechanical and thermal stimuli. We demonstrate that the same viral system can be used to deliver halorhodopsin (NpHR) to unmyelinated nociceptors, and

show that yellow light illumination can significantly raise mechanical and thermal withdrawal thresholds in these mice.

Disclosures: S.M. Iyer: None. **K.L. Montgomery:** None. **C.L. Towne:** A. Employment/Salary (full or part-time):; Circuit Therapeutics. **S.Y. Lee:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Circuit Therapeutics. F. Consulting Fees (e.g., advisory boards); Circuit Therapeutics. **S.L. Delp:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Circuit Therapeutics. F. Consulting Fees (e.g., advisory boards); Circuit Therapeutics.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.06/YY3

Topic: D.08. Pain

Support: CIHR MOP 86527

Louise and Alan Edwards Foundation

Title: Optogenetic silencing of peripheral pain pathways in transgenic mice

Authors: *I. DAOU[1], A. R. ASE[1], J. S. WIESKOPF[2], J. S. MOGIL[2], P. SEGUELA[1]; [1]Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada; [2]Dept. of Psychology, McGill Univ., Montreal, QC, Canada

Abstract: Nociceptors are primary afferent neurons activated by noxious stimuli. Their peripheral nerve endings are the sites of nociceptive transduction, therefore their selective activation and/or silencing can control pain perception. Using a tissue-restricted genetic strategy, we sought to generate an analgesic model in which pain is optically inhibited by silencing the activity of nociceptors using light-gated inhibitory opsins. This approach consisted of expressing the proton pump archeorhodopsin (ArchT) fused to EGFP in the Nav1.8+ nociceptors, using the Nav1.8-Cre recombinase driver line. Cellular distribution of the ArchT-EGFP construct was assessed in fluorescence in dorsal root ganglia (DRG), trigeminal ganglia, sciatic nerve, glabrous skin and dorsal horn of the spinal cord, and showed a strong and selective expression of ArchT in nociceptive soma and fibers. The strong labeling in laminae I and II of the dorsal horn and the glabrous skin demonstrated the efficient trafficking of ArchT from cell soma to the central and peripheral targets, making it a promising candidate for optical silencing of the peripheral pain

pathways. Electrophysiological recordings on cultured DRG neurons revealed significant outward photocurrents and hyperpolarizations in ArchT-expressing cells in response to yellow light (589 nm) stimulation. These light-evoked hyperpolarizations were sufficiently large to block electrically- as well as αβmeATP-induced action potentials. Behavioral analysis of the analgesic effects of yellow light in freely moving transgenic mice is in progress as sensory phenotyping is conducted under acute, inflammatory and neuropathic pain conditions, to determine whether ArchT activation can reduce/eliminate acute and/or chronic pain. The development of a model in which pain can be optically and remotely inhibited has the potential to lead to the discovery of novel optogenetic treatments for intractable chronic pain in humans.

Disclosures: I. Daou: None. **A.R. Ase:** None. **J.S. Wieskopf:** None. **J.S. Mogil:** None. **P. Seguela:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.07/YY4

Topic: D.08. Pain

Support: NSC 101-2320-B-182-040-MY3

CMRP180523

Title: Neuropeptide FF receptor type 2 transgenic mice exhibit hyperreactivity to nociceptive stimulation

Authors: *Y.-T. LIN[1], H.-L. LIU[2], H.-Y. LI[3], J.-C. CHEN[1];
[1]Grad. Inst. of Biomed. Sci., [2]Med. Imaging and Radiological Sci., Chang Gung Univ., Kwei-Shan, Taiwan; [3]Sci., Oregon Inst. of Technol., Klamath Falls, OR

Abstract: Neuropeptide FF (NPFF) belongs to FMRF-NH2 peptide and was known as an opioid modulating peptide. Two NPFF receptor subtypes have been cloned, i.e. NPFFR1 and NPFFR2. According to receptor competition assay, NPFFR2 was suggested to be physiological receptor for NPFF. NPFF-NPFFR system regulates morphine-induced analgesia and involves in both pro-nociceptive and anti-nociceptive responses, however the cellular mechanism remained unclear. The aim of this study was to explore if pain modulating effect of NPFF would be mediated through NPFFR2, taking advantage of in-house made NPFFR2 transgenic (Tg) mice. Behaviorally, NPFFR2 Tg mice exhibit hyperreactivity to mechanical and thermal nociceptive stimulations as compared to wild type (WT) mice. After CFA- or carrageenan-induced hind paw

inflammation, NPFFR2 Tg mice displayed a more severe allodynia than WT mice. Consistent with these findings, we demonstrated levels of NPFF and NPFFR2 mRNA in the lambar dorsal spinal were up-regulated after injection of CFA or carrageenan cord of WT mice. Via immunohistochemical analysis, we found protein levels of CGRP were significant increased after CFA injection in the NPFFR2 Tg mice as compared to CFA-treated WT mice. Further, functional MRI with electrical stimulation was introduced to evaluate brain activity in these mice and results showed that both signal intensity and activated extents in various brain regions (sensory cortex, thalamus, PAG, etc.) were much greater in NPFFR2 Tg mice than in WT mice. We conclude that NPFFR2 over-expression enhances the nociceptive threshold, thus plays an important role on pain modulation.

Disclosures: Y. Lin: None. **H. Li:** None. **J. Chen:** None. **H. Liu:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.08/YY5

Topic: D.08. Pain

Title: Validation of tonic painful EEG during cold pressor test

Authors: *M. GRAM, C. GRAVERSEN, S. S. OLESEN, A. E. OLESEN, A. M. DREWES; Medicinal Gastroenterology, Mech-Sense, Aalborg, Denmark

Abstract: Pain in experimental studies has traditionally been assessed by short stimuli to induce event-related potentials. The use of short stimuli however can be problematic, since they fail to accurately simulate clinical pain in the experimental setting. Tonic pain can be simulated using the cold pressor test and have been utilized both for induction of DNIC as well as EEG investigations of pain processing. Despite its growing use in EEG studies, no studies have so far investigated the inter-session reliability of EEG parameters during cold pressor pain. This study is aimed to investigate the reliability of EEG during the cold pressor test, and compare it to EEG during rest.
EEG was recorded from 40 healthy volunteers, on two separate days. Recordings were obtained both during rest and the cold pressor test using a 64-channel setup. The EEG signals were processed using the continuous wavelet transform to obtain the spectral distribution in 8 frequency bands, ranging from 1 to 70 Hz. Reliability between the two days was assessed using the coefficient of variation and the intra-class correlation.
The analysis showed excellent reliability for the resting EEG with intra-class correlation

coefficients above 0.8 and coefficients of variance below 15% for all frequency bands. Furthermore, the EEG during the cold pressor test proved to be comparable to the resting EEG with regards to reliability, with intra-class correlation coefficients above 0.9 and coefficients of variance below 15 % for all frequency bands.
This study found the inter-session reliability of EEG recorded both during rest and the cold pressor test to be excellent. This is an important validation step for previous and future EEG studies planning to utilize the cold pressor test for assessment of pain processing.

Disclosures: M. Gram: None. **C. Graversen:** None. **A.E. Olesen:** None. **A.M. Drewes:** None. **S.S. Olesen:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.09/YY6

Topic: D.08. Pain

Support: NIDA R01-DA033342A

Title: Neuropathic pain and the PFC: Dysregulation of the intercellular signaling pathways regulating pyramidal neuron excitability

Authors: *B. HARLAN[1], H. HUGHES[1], R. WANG[2], T. SHIPPENBERG[3], A. RIEGEL[1]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]IUPUI, Indianapolis, IN; [3]NIH, Bethesda, MD

Abstract: In MRI studies of humans with neuropathic pain, hyperexcitability in the dorsal medial Prefrontal Cortex (dmPFC) is correlated with a negative affective state. To better understand the cellular mechanisms that may drive this hyperexcitability, various behavioral, anatomical and electrophysiological measurements were employed at 3-30d following spared nerve injury (SNI). In behavioral tests, allodynia was maximal at 4d post-SNI. At >7d post-SNI, C-Fos expression in CaMKII positive, not GAD positive, dmPFC pyramidal cells was elevated and remained so thereafter. At 30d post-SNI, confocal analysis of L5 dmPFC pyramidal neuron dendrites labeled with the lipophilic dye (DiI) indicated an overall increase in the density of thin spines and filopodia , suggesting a persistent reorganization of dendritic structure and altered structural plasticity. In patch clamp recordings in brain slices from SNI animals, such neurons displayed a loss of the slow after-hyperpolarization (AHP) and a loss of inhibitory accommodation. This adaptation was most prominent in dmPFC neurons contralateral to the SNI limb and absent in surrounding brain regions. Accommodation could be restored to SNI tissue by: blockade of PKA with H89, depletion of intracellular Ca2+ with cyclopiazonic acid,

chelation of intracellular Ca2+ with BAPTA or bath application of retigabine, a stabilizer of KCNQ ion channels. In pharmacologically isolated voltage-clamp experiments, KCNQ currents activated by slow voltage ramps or depolarizing pulses rapidly inactivated via a mechanism involving excessive release of calcium from intracellular stores. Taken together these data suggest that SNI: (i) induces a persistent and rapid allodynia consistent with the sensory perception of pain, (ii) a slow to develop reorganization of structural plasticity and (iii) increases in neuronal excitability in specific dmPFC neurons via Ca2+ mediated-changes in the signaling of KCNQ ion channels.

Disclosures: B. Harlan: None. **H. Hughes:** None. **R. Wang:** None. **T. Shippenberg:** None. **A. Riegel:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.10/YY7

Topic: D.08. Pain

Title: Electrophysiological characterisation of projection neurons in naive and nerve-injured rats

Authors: *D. ROBERTS, H. REES;
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Abstract: Quantitative sensory testing (QST) is an established and clinically relevant tool used to diagnose the changes in somatosensory function underlying chronic pain conditions. QST protocols can assess and quantify both the sensory loss (negative) and sensory gain (positive) symptoms that characterise neuropathic pain. One such protocol developed by the German Research Network on Neuropathic Pain (DNFS) uses a mechanism-based system that is well characterized and with good test-retest and interobserver reliability.
Preclinical studies in animal models of nerve injury typically only study positive symptoms such as tactile allodynia. In contrast, extensive studies by the DFNS has revealed that neuropathic pain patients are just as likely to suffer from negative symptoms recording a lack of sensation. Using in vivo electrophysiology we aimed to study whether both the negative and positive sensory phenomena could be recorded in rats following nerve injury (spinal nerve ligation (SNL)). To do this we characterised the response properties of projection neurons in both naive and nerve-injured rats to avoid a selection bias. As far as possible the same stimuli used by the DFNS protocol were employed (i.e. cotton wool, brush, Von Frey hairs (2 - 60g), PinPrick stimulators (8 -512 mN), mechanical wind up (256 mN PinPrick stimulator) and thermal ramps (2°C, 42°C,

44°C, 46°C, 48°C and 50°C, Medoc TSA II).
Projection neurons were reliably identified using antidromic protocols. Preliminary data analysis also revealed that the majority of the identified projection neurons studied in naive and SNL rats responded only to high threshold stimuli. Strong mechanical stimuli were required such as Von Frey hairs (>15g), Pinprick stimulators, pinch and higher temperature stimuli (>48°C). A few neurones from SNL rats with ongoing baseline activity were also identified and studied. Studies detailing the characterisation of older rats as well as the pharmacology of projection neurons in SNL rats are also described.

Disclosures: D. Roberts: None. **H. Rees:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.11/YY8

Topic: D.08. Pain

Title: Application of Mdr1a/b-Bcrp knockout mice to evaluate role of CNS penetration to antinociceptive efficacy

Authors: *S. K. JOSHI, C. ZHU, L. LEWIS, C. ZHONG, D. GAUVIN, J. MIKUSA, C. ZHAN, C. KALVASS, A. BANNON;
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Abstract: In analgesic drug discovery an important consideration for any given target is the relevance of central nervous system (CNS) penetration for efficacy. The P-glycoproteins (Pgp) expressed in the endothelial cells of the blood brain barrier extrude drugs from the CNS thus limiting their CNS concentration. The Mdr1a/b-Bcrp triple knockout mice have targeted mutations of the major such P-glycoproteins. In the present experiments, we first tested whether the knockout mice exhibit a normal pain phenotype by performing testing in acute (thermal, mechanical), inflammatory (complete Freund's adjuvant, CFA) and neuropathic (spinal nerve ligation, SNL, and chronic constriction injury, CCI) pain models. We then evaluated efficacy of A-1048400, a Pgp-substrate in wild type (WT) and knockout (KO) mice.
In agreement with previous reports, the Pgp KO mice were viable and appeared normal as demonstrated by comparable body weight gain and locomotor activity compared to WT mice. The acute pain responses as well as development and maintenance of nociceptive hypersensitivity in CFA (thermal and mechanical) and SNL (mechanical) models were comparable in the WT and KO mice. In WT mice A-1048400 produced efficacy at 30 and 100

mg/kg in the CCI model. In KO mice, A-1048400 produced significant antinociceptive effects at a lower dose of 10 mg/kg. In WT mice mean brain levels at the 10 mg/kg dose were 10 ± 3 ng/g while in KO mice mean brain levels were 110 ± 14 ng/g. These data provide evidence that CNS penetration can enhance the efficacy of A-1048400. Overall, these data highlight the utility of using Mdr1a/b-Bcrp triple knockout mice as a tool for assessing the role of CNS penetration for contributing to effects of novel compounds.
Disclosures: All Authors are AbbVie employees. The design, study conduct, and financial support for the investigation were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the abstract.

Disclosures: S.K. Joshi: A. Employment/Salary (full or part-time):; AbbVie, Inc. **C. Zhu:** A. Employment/Salary (full or part-time):; AbbVie, Inc. **L. Lewis:** A. Employment/Salary (full or part-time):; AbbVie Inc. **C. Zhong:** A. Employment/Salary (full or part-time):; AbbVie Inc. **D. Gauvin:** A. Employment/Salary (full or part-time):; AbbVie Inc. **J. Mikusa:** A. Employment/Salary (full or part-time):; AbbVie Inc. **C. Zhan:** A. Employment/Salary (full or part-time):; AbbVie Inc. **C. Kalvass:** A. Employment/Salary (full or part-time):; AbbVie Inc. **A. Bannon:** A. Employment/Salary (full or part-time):; AbbVie Inc.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.12/YY9

Topic: D.08. Pain

Support: Wellcome Trust Investigator Award

Title: Characterising the role of Langerhans cells in models of chronic pain conditions

Authors: *M. THAKUR, F. DENK, S. MCMAHON;
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Abstract: Langerhans cells are a self-sustaining epidermal population of dendritic cells with functions including the phagocytosis of antigens in the epidermis and maintenance of immune tolerance. There is circumstantial evidence that they are involved in the pathogenesis of certain chronic pain disorders.
We have used a knock-in mouse expressing human diphtheria toxin receptor in langerin-expressing cells to probe interactions between Langerhans cells and peripheral nerve in a number of models of chronic pain conditions. We find that epidermal Langerhans cells cannot be detected in sections of hindpaw skin for at least 18 days following two i.p doses of 1µg

diphtheria toxin.
In pilot studies we assessed how ablation of Langerhans cell ablation affects the pathophysiology of the Seltzer model of traumatic nerve injury-induced pain. Epidermal C-fibre terminals were found to be cleared less rapidly after nerve injury in Langerhans-depleted mice, suggesting a novel role for these cells in axonal phagocytosis during Wallerian degeneration.

Disclosures: M. Thakur: None. **F. Denk:** None. **S. McMahon:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.13/YY10

Topic: D.08. Pain

Support: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministiry of Education, Science and Technology (2012008491)

Title: Modulation of pain by wnt signaling molecules in an experimental neuropathic pain model

Authors: J.-A. YOON, K. SUNG, *S. LEE;
Dept Physiol, Col. Med., Hanyang Univ., Seoul, Korea, Republic of

Abstract: Wnt signaling molecules are important in development and differentiation of nervous tissues. Recently, a few studies have suggested that wnt signaling might contribute to development and maintenance of pathological chronic pain. However, direct evidences that enhancement of wnt signals might actually induce painful behavior are very limited. This study was designed to examine that stimulation or inhibition of wnt signaling could modulate pain behaviors in experimental animals. Pain was assessed by mechanical threshold evoking withdrawal response (PWT) or paw withdrawal latency (PWL) to thermal stimuli. Agonists or antagonist of wnt signaling were injected into the spinal cavity of normal or neuropathic rats through intrathecal catheters, and their PWT or PWL were measured before and after the injections. Intrathecal injection of a wnt agonist elicited hyperalgesic signs, the reduction of PWT, indicating that increase of Wnt signaling might be to develop hyperalgesic signs. The hyperalgesic effect was completely blocked by peremptive antagonist injection. Intrathecal injection of a Wnt3 antagonist increased PWT of rats suffering neuropathic pain, induced by L5 & 6 spinal nerve transection. These results suggest that increase of wnt signaling might be one of the factors which change the spinal plasticity, leading the hypersensitivity to stimuli. However, the mechanisms of pain modulation of wnt signaling are still obscure.

Disclosures: J. Yoon: None. **K. Sung:** None. **S. Lee:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.14/YY11

Topic: D.08. Pain

Support: the Industrial Source Technology Development Program (no. 10033812) of the Ministry of Knowledge Economy (MKE) of Korea

the Smart IT Convergence System Research Center (no.2011-0031867) funded by the Ministry of Education, Science and Technology.

Title: Modulation of neuronal hyperactivity in ventral posterolateral nucleus by electrical stimulation of anterior cingulate cortex in neuropathic pain model rats

Authors: *S. RYU[1], J. CHOI[1], J. KIM[2], C. IM[3], J. CHANG[2], H.-C. SHIN[3], K. KIM[1]; [1]Biomediecal Engin., Yonsei Univ., Wonju, Kangwon, Korea, Republic of; [2]Dept. of Neurosurg. and Brain Res. Inst., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [3]Dept. of Physiol., Col. of Medicine, Hallym Univ., Chuncheon, Korea, Republic of

Abstract: Electrical stimulation to a specific area in the brain has been widely used to treat neurological disorders such as Parkinson's disease. It has been recently reported that electrical stimulation to anterior cingulate cortex (ACC) is effective to relieve symptoms of neuropathic pain. To understand its mechanism, it is necessary to observe how neuronal activities in the pain-related area are affected by the stimulation. In this study, we investigated pain-related characteristics of neuronal activities in ventral posterolateral nucleus (VPL) of thalamus and analyzed how they are affected by ACC electrical stimulation. Spared nerve injury rats (200-300 g) were used as models of neuropathic pain.Singe unit activities and local field potentials (LFPs) were recorded from the VPL. Press stimulus was applied to the hind paw. Biphasic electrical pulse was applied to an electrode placed in the ACC (pulse amplitude: 100 μA, pulse duration: 60 μS, frequencies of 10-130 Hz). In single unit activities, firing rate was increased during the press stimulation both in normal and neuropathic pain model rats. However, firing rate was increased significantly higher and lasted much longer (after-discharge) in neuropathic pain model than in the normal rats. According to the press stimulus, spectral power of LFPs was increased in high gamma band (80-150 Hz) and decreased in low frequency band (1-10 Hz). Similarly to the single unit activities, changes in LFPs also were sustained longer in neuropathic pain model than in the normal rats. By the electrical stimulation of the ACC, the hyperactivity in

single unit activities including the after-discharge was suppressed. The duration of increased/decreased LFP spectral powers were also reduced to near-normal levels by the ACC stimulation. The ACC stimulation became effective when the stimulation frequency was higher than 50 Hz. Our results suggest that the abnormal singe unit activities and LFPs are distinctive features of neuropathic pain, and high frequency electrical stimulation of the ACC is an effective way to reduce these pain-specific features.

Disclosures: S. Ryu: None. **J. Kim:** None. **C. Im:** None. **J. Chang:** None. **H. Shin:** None. **K. Kim:** None. **J. Choi:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.15/YY12

Topic: D.08. Pain

Support: R01NS62306

R03NS077193

Title: Pharmacological characterization using Ca2+ imaging in *Ex vivi* spinal cord slices during inflammatory or multiple sclerosis pain

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Abstract: We recently reported successful imaging of multiple neurons in adult spinal cord slices, and found that SNI increases glutamate-evoked Ca2+ mobilization. Here we extend this characterization to pharmacological studies in animal models of inflammation and multiple sclerosis (MS).
To test the hypothesis that Ca2+-dependent central sensitization is maintained not only during but also after the resolution of inflammatory hyperalgesia, we used live-cell Ca2+ imaging in adult mouse spinal cord slices. von Frey (vF) measures of tactile sensitivity were assessed at 3 d, 7 d or 21 d after CFA (5 ul, intraplantar). Transverse lumbar spinal cord slices (450 um) were bulk loaded with 10 uM fura-2 AM for ratiometric Ca2+ imaging. Glutamate-evoked [Ca2+]i in

lamina II dorsal horn neurons was potentiated at 3 d after CFA ($p<0.05$) and then resolved by day 21; this coincides with the temporal onset and resolution of inflammatory hyperalgesia. Perfusion of either the selective mu antagonist, CTOP or the non-selective opioid receptor antagonist NTX markedly increased glutamate-evoked [Ca2+]i in CFA 21d ($p<0.05$) but not sham slices. Similarly, opioid receptor blockade 21d post-CFA reinstated pain-like behavior. These data suggest endogenous opioids mask Ca2+-dependent central sensitization and pain-like behavior 21d after CFA-induced inflammation.
To test the hypothesis that the MS-induced pain is associated with glutamate-evoked Ca2+ responses, we evaluated the glutamate dose-response relationship of Ca2+ mobilization 7-10 days after induction of experimental autoimmune encephalomyelitis (EAE). EAE was induced in 24 d mice by injecting myelin oligodendrocyte glycoprotein 35-55 (MOG33-35; 300 ug) and again 6 d later. Pertussis toxin was administered (200 ng, i.p.) on days 0 and 2. Prior to (baseline) and up to 21 d post-injury, tactile (vF) and cold (acetone) hypersensitivity were assessed. EAE clinical scores and pain-like behavior were significantly greater compared to control animals by 7 d after EAE induction. EAE significantly increased Ca2+ responses to 0.3 and 1 mM glutamate ($p<0.05$). We next tested the hypothesis that FTY720, a sphingosine-1-phosphate ligand that is FDA approved for the motor dysfunction of MS, reduces the central sensitization that drives tactile and cold allodynia. FTY720 (1 mg/kg i.p.) daily beginning 6 days after the first MOG injection significantly reversed tactile and cold allodynia compared to saline-injected controls; and Ca2+ responses to 0.3 and 1 mM glutamate were also significantly lower ($p<0.05$). These data suggest a functional link between Ca2+ signaling in the dorsal horn and the maintenance of inflammatory and MS-induced pain.

Disclosures: S. Doolen: None. **T. Iannitti:** None. **B.K. Taylor:** None. **G. Corder:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.16/ZZ1

Topic: D.08. Pain

Title: Characterization of nociceptive sensitization in larval Manduca sexta

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Abstract: Nociceptive sensitization results when an animal learns to strengthen its defensive response to a previously neutral or weak stimulus after the presentation of a noxious or painful stimulus such as a strong pinch or an electrical shock. This phenomenon has been observed in the hornworm, *Manduca sexta*, by testing the number of defensive strikes that occurs before and after a strong pinch (Walters et al., 2001). We have been developing a novel approach to quantifying nociceptive sensitization, while concurrently using Walter's method to assess the role that cGMP plays in this sensitization. Our new method assessed the force needed to generate a strike before and after the aversive stimulus, and our data indicated that larvae were sensitive to a lighter touch after presentation of the noxious stimulus, which lasted for over 18 hours. Our pharmacological study suggested that cGMP has an antinociceptive effect. Injection of 8-bromo-cGMP attenuated the sensitization induced by a strong pinch in a dose-dependent manner, as noted by a decreased number of strikes after cGMP application. In contrast, strike number increased after injection of methylene blue, a non-specific inhibitor of soluble guanylyl cyclase activity used to inhibit the production of cGMP, although no effect was noted with ODQ, an inhibitor of NO-sensitive cGMP activity. These data suggest that an alternate transduction pathway sensitizes the strike response, and cAMP is being assessed currently.

Disclosures: M. Fuse: None. **E. Merchasin:** None. **M. McMackin:** None. **K. Iwasaki:** None. **L. Ramos:** None. **C. Moffatt:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.17/ZZ2

Topic: D.08. Pain

Support: NIH Grant DA011471

NIH Grant CA091007

NIDA Grant T32 DA07234

Title: Chronic administration of the cannabinoid agonist, WIN 55,212-2, reduces hyperalgesia and nociceptor sensitization produced by cisplatin

Authors: *M. L. UHELSKI[1], C. HARDING-ROSE[2], D. SIMONE[2];
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Abstract: Painful chemotherapy-induced peripheral neuropathy (CIPN) is the major dose-limiting side effect of chemotherapy, which could impact the efficacy of therapy for patients who are unable to tolerate increasing doses. Many patients continue to experience neuropathic symptoms for months or years after treatment has ended. Neuropathic pain due to chemotherapy has been explored in animal models. The platinum compound cisplatin produces mechanical, heat, and cold hyperalgesia in rodents and is associated with damage to primary sensory neurons. Cisplatin has been shown to sensitize nociceptors, evidenced by an increase in spontaneous discharge among both Aδ- and C-fiber nociceptors and an increase in evoked responses. Cannabinoids have been shown to reduce neuropathic pain, including pain from CIPN. Here we examined the effects of chronic administration of the non-selective cannabinoid receptor agonist WIN 55,212-2 on hyperalgesia and sensitization of nociceptors produced by cisplatin. Mice received a daily injection of WIN 55,212-2 (2 mg/kg, ip) or vehicle with cisplatin (1 mg/kg, ip) for 7 consecutive days. We found that WIN 55,212-2 attenuated the development of mechanical and heat hyperalgesia associated with cisplatin administration. Twenty-five percent (2/8) of Aδ- and 76% (13/17) of C-fiber nociceptors exhibited ongoing spontaneous discharge in mice pretreated with vehicle, whereas only 15% (2/13) of Aδ-fibers and 38% (8/21) of C-fiber nociceptors were spontaneously active in mice given WIN 55,212-2. Nociceptors that were spontaneously active in the latter group had lower mean discharge rates for both Aδ- (0.3 ± 0.3 Hz vs 0.1 ± 0.1 Hz) and C-fibers (0.2 ± 0.0 vs. 0.1 ± 0.7 Hz). There was also a decrease in responses of Aδ-fibers evoked by a mechanical stimulus (von Frey monofilament with 147 mN force). Responses in mice given cisplatin with vehicle or WIN 55,212-2 were 91.9 ± 21.4 vs. 64.9 ± 14.3 impulses, respectively. Evoked responses of C-fiber nociceptors did not differ between the groups. These data demonstrate that daily administration of cannabinoids reduces hyperalgesia and nociceptor activity produced by cisplatin and may be useful to reduce pain from chemotherapy treatment.

Disclosures: M.L. Uhelski: None. **C. Harding-Rose:** None. **D. Simone:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.18/ZZ3

Topic: D.08. Pain

Support: NIH AR047410 (KEM)

Title: Vesicular Glutamate Transporter 2 and Aspartate Aminotransferase alterations in rat DRG neurons during adjuvant-induced arthritis

Authors: *B. BOLT, K. E. MILLER;
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Abstract: The sensory neurons of the dorsal root ganglia (DRG) are glutamatergic, utilizing glutaminase (GLS) and aspartate aminotransferase (AST) for glutamate synthesis (Miller et al., Pharmacol Ther 130:283, 2011). Following synthesis, glutamate is transported into synaptic vesicles by either vesicular glutamate transporter (VGLUT) 1 or 2 (Brumovsky et al., Neurosci 147:469, 2007). VGLUT2 is produced primarily in medium (400-800 μm2) to small sized (100-400μm2) DRG neurons, whereas large sized DRG neurons (>800 μm2) predominantly express VGLUT1. We previously demonstrated that glutamate metabolism (↑ GLS, AST, glutamate levels) is altered in the rat DRG, sciatic nerve and skin during adjuvant-induced arthritis (AIA). Additionally, we have determined that AST is elevated in nociceptive, calcitonin gene-related peptide (CGRP) containing DRG neurons during AIA. In the present study, we evaluated alterations of VGLUT2 in AST DRG neurons and AST in VGLUT2 DRG neurons during AIA. Complete Freund's adjuvant was injected into the rat right hindpaw to induce AIA and DRG's were analyzed at 1, 2, 4 and 8 days. Rats were anesthetized and transcardially perfused with fixative. AST and VGLUT2 colocalization was evaluated in L4 DRG's with immunohistochemistry, followed by quantitative image analysis with Image J. At 1, 2 and 8 days AIA, AST-immunoreactivity (ir) in VGLUT2-positive DRG neurons was elevated when compared to naïve controls. Furthermore, VGLUT2-ir in AST-positive neurons was elevated slightly at day 1 AIA, but at no other time points when compared with controls. These results suggest that part of the alteration of glutamate metabolism during peripheral inflammation is due an increase in VGLUT2 and AST levels in DRG neuronal cell bodies. The underlying mechanisms for these alterations could be due to retrograde transport of neurotrophic factors and/or modification of transcriptional, translational, or trafficking processes in the DRG neuronal cell body.

Disclosures: B. Bolt: None. **K.E. Miller:** None.

Poster

558. **Pain Models: Physiology**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 558.19/ZZ4

Topic: D.08. Pain

Support: Canadian Institute of Health Research (CIHR)

National Research Fund of Luxembourg (FNR: AFR-PDR-1002974)

Title: Effects of distraction and suggestions with and without hypnotic induction on spinal-motor, autonomic and cortical pain-evoked responses

Authors: *A. STREFF[1,2], B. HOUZE[1,2], A. LEHMANN[1,4,2], M. PICHE[1,5], P. RAINVILLE[1,3]; [1]psychology, CRIUGM, Montreal, QC, Canada; [3]Stomatology, [2]Univ. de Montreal, Montreal, QC, Canada; [4]BRAMS, Montreal, QC, Canada; [5]Univ. de Trois-Rivieres, Trois-Rivieres, QC, Canada

Abstract: While the effects of hypnotic suggestions on pain are well-known, the specific contribution of "hypnotic states" in acute pain modulation is less described. At what level and how exactly hypnosis changes nociception? Using psychophysiological and electroencephalographic measures, this study examines a modulation of pain-related responses elicited by noxious electrical stimulation induced by four manipulated conditions: hypo- and hyperalgesic suggestions after induction, hypoalgesic suggestions without induction and a mental distraction task.

10 women and 10 men (age: Mn= 25±7 yrs.) participated in two sessions (hypnosis vs. non-hypnosis; duration 90-120 min.) with two experimental parts. Sessions and parts were counterbalanced with respect to order and gender. Noxious electrical shocks (30-ms train of 1-ms pulses at 333 Hz; ISI = 6-12s) were administered to the right sural nerve, at an intensity of 120% of the individual RIII-reflex threshold determined in a pre-experimental block. In each session, stimuli were administered in 3 blocks of 6 shocks after neutral suggestions and 2 blocks of 9 shocks after hypo- (with and without induction) and hyperalgesic suggestions or during the calculation task. After each block, participants rated pain intensity and unpleasantness. Electromyography (EMG) of the right biceps femoris, electrocardiographic activity (ECG) and skin conductance responses (SCR) were monitored continuously. Additionally, electroencephalographic activity (EEG) was recorded in order to investigate late SEPs (N150-P260 components) in each condition.

The four experimental conditions modulated pain intensity ratings ($p=0.001$) compared to the neutral condition. Hypoalgesic suggestions significantly decreased RIII-reflex amplitude with ($p<0.001$) and without induction ($p=0.009$) whereas hyperalgesic suggestions increased EMG activity ($p<0.001$). SCRs and heart rate were reduced after hypo- ($p=0.008$; $p=0.001$) and increased after hyperalgesic ($p= 0.004$; $p=0.006$) suggestions. The mental calculation task only accelerated the cardiac rhythm ($p<0.001$). P260 component was significantly reduced on Cz ($p=0.025$) and Pz ($p=0.008$) locations only after hypoalgesic suggestions preceded by hypnotic induction.

Hypoalgesic suggestions, after induction only, modulate pain ratings, motor, autonomous and cerebral responses. These results indicate that different psychological strategies used to modulate pain act through at least partly separable mechanisms. Further experiments should include BOLD measures to get a more detailed idea of the brain regions involved in the modulation of a spinal nociceptive reflex by hypnosis.

Disclosures: A. Streff: None. **B. Houze:** None. **A. Lehmann:** None. **M. Piche:** None. **P. Rainville:** None.**Poster**

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.01/ZZ5

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Computational model of in-vitro sigh generation

Authors: *N. TOPORIKOVA[1], A. ZAIDI[2], M. THOBY-BRISSON[3];
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Abstract: Eupneic breathing in mammals is periodically interrupted by spontaneous augmented breaths (sighs) that are characterized by a biphasic larger-amplitude inspiratory burst followed by post-sigh apnea. Previous in vitro studies in newborn rodents have demonstrated that the respiratory oscillator of the pre-Bötzinger complex (preBötC) can generate the distinct inspiratory-related motor patterns for both eupnea- and sigh-like activity. However it remains debated whether these two types of inspiratory activities are produced by the same neuronal population or by distinct sub-networks. In order to address this issue we developed two mathematical models to investigate possible mechanisms for sigh generation. One model proposes that sighs are generated by two distinct neural sub-networks, while the second model hypothesizes that sighs are generated within the same network, with sigh generation relying on calcium-dependent mechanisms in both cases (as suggested by Lieske et al., 2000; Pena et al., 2004 and Thoby-Brisson et al, unpublished). For each model we searched for parameters (such as synaptic strength, pacemaker properties, distribution on ionic currents) that allow reproducing experimental data obtained in vitro on mouse transverse brainstem slices isolating the preBötC. Although the low frequency dynamics of the sigh and its biphasic shape can be reproduced by both models, decoupling of the two components of a sigh event after blockade of glycinergic synapses (Lieske et al., 2000) was only achieved in the two sub-network model. This model also simulated the increase in sigh frequency in response to Gq-coupled neuromodulator (Tryba et al., 2008) and independence of the sigh frequency from the extracellular K+ concentration (Thoby-Brisson et al., unpublished) Overall, our data suggest that sigh generation mechanisms rely on the activity of a specific sub-population of neurons within the preBötC network.

Disclosures: N. Toporikova: None. **A. Zaidi:** None. **M. Thoby-Brisson:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.02/ZZ6

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR-229985

FRQS-RSBO-5213

Title: Astrocytes determine conditions for rhythmogenesis in a trigeminal sensori-motor circuit involved in mastication

Authors: *P. C. MORQUETTE[1], D. VERDIER[1], R. ROBITAILLE[1], A. KOLTA[2];
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Abstract: In trigeminal and spinal circuits thought to be involved in mastication and locomotion respectively, rhythmic neuronal bursting is enhanced and promoted when the extracellular concentration of calcium [Ca2+]e decreases. This study investigates the ability of astrocytes to modulate neuronal bursting by influencing the [Ca2+]e in the trigeminal main sensory nucleus (NVsnpr). In vitro, neurons of the dorsal NVsnpr fire rhythmic bursts spontaneously when Ca2+ is removed from the perfusing medium, or when a calcium chelator like BAPTA is applied locally next to the recorded cell. Under physiological [Ca2+]e (1.6mM), local applications of NMDA or repetitive stimulation of afferent inputs to NVsnpr can also elicit rhythmic bursting. We used Ca2+ -imaging and whole cell patch recordings to test whether astrocytes respond to these “burst inducing stimuli”. Both stimuli induced large depolarizations in recorded astrocytes. This activation was further confirmed for NMDA applications using Ca2+ -imaging with either intracellular or extracellular Ca2+ indicators. When the latter were used, NMDA application invariably caused activation of multiple cells. In dual recordings of pairs of neurons and astrocytes, the astrocytic depolarization occurred in parallel to rhythmic neuronal bursting. To determine the presence or absence of causality in this relationship, we took advantage of the fact that astrocytes connect through gap junctions to selectively prevent their activation by patching a single one with a pipette filled with BAPTA and letting the BAPTA diffuse. After allowing 30-50 min for BAPTA diffusion, the inactivation of the glial syncytium was confirmed by the extinction of Ca2+ responses in surrounding astrocytes. In paired recordings NMDA could no longer induce bursting in neurons recorded under this condition, despite maintaining its ability to depolarize them. However, inactivation of glial syncytium had no effects on the spontaneous rhythmic bursting of neurons recorded in calcium free ACSF. Thus direct decrease of [Ca2+]e seemed to bypass the astrocytic implication, suggesting that the effect of astrocytes is mediated through an effect on [Ca2+]e .

Interestingly S100β, a calcium binding protein that has been shown to be secreted from astrocytes could act as a Ca2+ chelator. Local application of S100β induces rhythmic bursting and pre-treatment of slices with an antibody against it prevents NMDA-induced bursting in 7/11 cases, whereas in 5/5 cases bursting was not prevented by use of an unspecific antibody. This work provides strong evidence that rhythmogenesis in a part of the masticatory CPG depends on neuron-astrocyte interaction.

Disclosures: P.C. Morquette: None. **D. Verdier:** None. **R. Robitaille:** None. **A. Kolta:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.03/ZZ7

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR 229985

FRQS-RSBO 5213

Title: Stimulation of the trigeminal sensory tract causes bursting and calcium depletion in dorsal neurons of the trigeminal main sensory nucleus

Authors: *P. A. KADALA, D. VERDIER, A. KOLTA;
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Abstract: Central pattern generators (CPGs) underlie rhythmic movements like breathing, walking and chewing. For the latter, a subpopulation of neurons located dorsally in the trigeminal main sensory nucleus (NVsnpr), may be the core of the masticatory CPG. Previous investigations in rats have shown that artificially lowering the concentration of extracellular calcium promotes rhythmic bursting in these neurons. A stimulation of the afferent sensory tract to the NVsnpr or local application of N-Methyl-D-Aspartate (NMDA) can also cause them to switch from a tonic firing to a bursting pattern. The question remains (i) whether or not the extracellular calcium fluctuates during stimulation of afferent sensory fibers and if any, what is the extent of that fluctuation, (ii) what are the mechanisms underlying these changes in extracellular calcium concentrations. Parallel work from our laboratory suggests that astrocyte activation is essential to neuronal bursting. The protein S-100β is secreted to extracellular space by astrocytes and has been shown to play numerous roles in the brain, including chelation of calcium. As such, it might cause calcium fluctuations in extracellular space. In the present study, we used calcium-sensitive electrodes to monitor changes in extracellular calcium concentration

along with stimulations of the sensory tract in brainstem slices obtained from rats. We also investigated the effect of local application of S-100β and an anti-S100β antibody on both the extracellular calcium concentration and the firing pattern of NVsnpr neurons. After stimulation of the sensory tract, 30% of neurons switched from a tonic (singlets or doublets) to a bursting firing pattern. Most of these changes occurred conjointly with a decrease in extracellular calcium concentration. Calcium decreases exhibited two profiles: a transient calcium depletion that was paralleled by a complete inhibition of neuronal firing, and a slow long-lasting decrease which was accompanied by a switch to bursting firing pattern. Application of S-100β (50 or 129 μM) caused neurons to burst and decreased extracellular calcium by ~1mM. Similar effects were obtained with NMDA (1mM). Pretreatment of the slices with an anti-S-100β antibody but not with a non-specific antibody, prevented burst induction and calcium depletion with stimulation of the sensory tract and application of NMDA or S-100β. Together, these data show that stimulation of trigeminal sensory tract can trigger depletion in extracellular calcium along with burst discharges in trigeminal main sensory nucleus and these effects are mediated by release of the astrocyte protein S-100β.

Disclosures: P.A. Kadala: None. **D. Verdier:** None. **A. Kolta:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.04/ZZ8

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS083319

DFG Grant DA1188

Title: The effect of ectopic axonal spiking on synaptic dynamics

Authors: *N. DAUR[1,2], Y. ZHANG[3], F. NADIM[2,3], D. BUCHER[1,2];
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Abstract: Information flow in neurons usually consists of synaptic integration and spike initiation in proximal compartments, and axonal propagation of signals to distal synaptic targets. However, distal (ectopic) axonal spike initiation has recently been shown to play a role in neural processing during specific network states in cortex and hippocampus.
We use an unmyelinated crustacean motor axon of the lobster stomatogastric nervous system,

and its postsynaptic muscle targets, to investigate the role of ectopic spikes in shaping postsynaptic responses. The rhythmically active Pyloric Dilator neurons send 4-5 cm long axons to different target muscles. We have previously shown that low concentrations of dopamine enhance I_h in these axons. The resulting depolarization can be sufficient to elicit ectopic peripheral spike initiation (Bucher et al., 2003; Ballo & Bucher, 2009, Ballo et al., 2010). The incidence of peripheral spike initiation depends on the level of centrally generated activity, as higher burst frequencies are sufficient to suppress peripheral spike initiation. Axonal stimulations with lower burst frequencies, as well as dampening of centrally generated activity by modulatory inputs lead to patterns of centrally generated bursts interleaved with a few peripherally generated spikes during the interburst intervals. These additional spikes have a "priming" effect on the postsynaptic muscle responses in that they enhance the compound depolarization in response to bursting input by 20-40 %. This is true even in muscle fibers that show no discernible direct response to the interburst spikes. Furthermore, the priming effect is not dependent on the overall spike numbers, but persists when overall spike rate is kept constant. The muscle responses appear largely passive, but current and voltage clamp recordings show some voltage-dependent non-linearity and occasional spike-like responses. However, the priming effect qualitatively persists when synaptic currents are measured. Therefore, the priming effect appears to be due to a mixture of short-term synaptic dynamics and active (threshold- or voltage-dependent) responses.
A mathematical decoding technique applied to these data indicates that a combination of fast depression and slow facilitation can account for the priming effect. However, when the same decoding process is applied to data from *Poisson*-like stimulations of the same muscle fiber, it suggests a different history dependence that fails to capture the priming effect. Together, these results confirm that the priming effect is not merely a history-dependent effect but also involves nonlinear voltage- or calcium-dependent processes.

Disclosures: N. Daur: None. **Y. Zhang:** None. **F. Nadim:** None. **D. Bucher:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.05/ZZ9

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS070583

NIH Grant NS066587

Title: Modulatory masking: Activation of distinct currents in a single cell supports temporary reversals of response character in a multi-functional network

Authors: *M. H. PERKINS, E. C. CROPPER, K. R. WEISS;
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Abstract: Repetition priming is an improvement in response quality to a stimulus that has been experienced. Underlying this phenomenon is an alteration of the state of the behavior-generating network. Many networks are multi-functional, and respond to stimuli with different, even antagonistic behaviors. In networks where two antagonistic behaviors are both subject to priming, how do transitions between behaviors occur? We used Aplysia feeding as a model system. The buccal and cerebral ganglia, which form a control network, generate responses that are state dependent. When rested, the network responds to a stimulus and generates intermediate programs, that are neither ingestive nor egestive. Following repeated stimulation of the interneuron CBI-2, responses are ingestive. In contrast, following repeated stimulation of the esophageal nerve, responses are egestive.
The ingestive vs. egestive character of motor programs is in part dictated by changes in the excitability of the motor neuron B48. We initially hypothesized that the network state would be manifested in B48 as a single variable with ingestive and egestive extremes. Further, we predicted that a transition from an ingestive to an egestive state would involve an erasure of the pre-existing increase in B48 excitability. Instead, we observed that the excitability increase in B48 caused by ingestive priming is transiently suppressed, but not erased, by subsequent egestive priming. In light of this finding, we test a hypothesis, that postulates that the network state depends on a dual-process mechanism. It depends on the degree of increased excitability due to ingestive priming and on the degree of decreased excitability due to egestive priming. Further, we test a simple model for achieving such dual-process control, in which two distinct currents are responsible for the excitability changes in B48. We find that neuropeptides that support ingestive priming (Cerebral Peptide 2, CP2 and Feeding Circuit Activating Peptide, FCAP) modulate a current that does not reverse between -100 and 0 mV, is reduced by removal of sodium, and potentiated by removal of extracellular calcium. A current with similar characteristics was observed, following the iontophoresis of cAMP. This suggests that ingestive priming increases the excitability of B48 via a cyclic nucleotide gated sodium current. A current with different characteristics seems to be involved in the reduction of B48 excitability following egestive priming. It reverses near -85 mV, is sensitive to TEA and to removal of calcium. We conclude that the sum-total network-state arises from integration of a dual-process mechanism at the cellular and sub-cellular level.

Disclosures: M.H. Perkins: None. **E.C. Cropper:** None. **K.R. Weiss:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.06/ZZ10

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: F31 NS 080420

NS 081013

Title: Long-term regulation of excitability in the gastric mill network of the crab stomatogastric ganglion

Authors: *A. W. HAMOOD, S. HADDAD, E. MARDER;
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Abstract: The stomatogastric ganglion (STG) of crustaceans contains the neural circuitry involved in the generation of two motor patterns critical for feeding behavior in the animal. The pyloric (filtering) rhythm, is continuously active both in vivo and in dissected in vitro preparations of the stomatogastric nervous system (STNS). The gastric mill (chewing) rhythm is episodically active and drives a set of teeth in the animal's stomach to perform grinding and chewing movements, typically activated by modulatory inputs only after feeding. Previous work has shown that upon removal of these modulatory inputs by transection of the only input nerve (decentralization), activity in both networks ceases, but the pyloric rhythm gradually returns over a period of several days in vitro. However, recovered activity in the gastric mill network has never previously been reported in decentralized preparations. We have performed long-term extracellular recordings from in vitro preparations of the STNS for a week or more, and find that rhythmic gastric mill activity can return in vitro in the absence of modulatory inputs. Bursting activity in gastric mill neurons was routinely observed and rhythmic bursting activity in gastric mill neurons was observed in more than half of these preparations, starting between 150 and 350 hours following decentralization. Intracellular recordings from the motor neurons of the gastric mill network both immediately after decentralization, and at later time points, including in the presence of recovered gastric mill rhythms show significant increases in neuronal excitability of gastric mill neurons following decentralization, suggesting that the regulation of intrinsic neuronal excitability is a key feature underlying recovery. We have also performed acute modulatory perturbations of the STNS during decentralization, and monitored the changing responsiveness to neuromodulation during the culture period, providing further insight into the changes in network excitability that accompany the observed network response to this dramatic perturbation.
Supported by F31 NS 080420 and NS 081013

Disclosures: A.W. Hamood: None. **S. Haddad:** None. **E. Marder:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.07/ZZ11

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH NCRR 5P20RR016463-12

NIH NIGMS 8 P20 GM103423-12

AV Davis Foundation

Title: Cloning and sequencing of prepropeptide cDNAs that encode neuropeptides involved in feeding-related behaviors in the pond snail, Helisoma trivolvis

Authors: *N. W. KLECKNER[1], C. O'LEARY[2], J. BERGERON[3], A. HULSE[3], M. ARSNOW[3], D. BIRKHEAD[4], G. BORLAND[2], P. DIXON[4], H. FISHER[4], C. GARVEY[3], J. MEYO[3], V. JARVIS[3], A. SRIDHAR[4], J. D. SATO[5];
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Abstract: Molluscan nervous systems have long been used as models for understanding the neural basis of behavior. The sea slug, *Aplysia californica*, has been used, for example, to understand signaling and circuits involved in learning and memory and the neuromodulation of feeding and other behaviors. Motor program switching among a variety of feeding-related behaviors in in *Aplysia* and freshwater mollusks is under the control of cerebral-buccal and buccal interneurons that release neuropeptides such as neuropeptide phenylalanine (NPF), APGWamide, small cardioactive peptide B (SCP_B) and gonadotropin releasing hormone (GnRH) onto central pattern generator (CPG) neurons that pattern feeding behavior. The purpose of these experiments was to identify and sequence cDNAs coding for prepropeptides from freshwater snails to better understand the distribution and roles of the mature peptides in pond snail species that serve as intermediate hosts for parasites. RNA was isolated from *Helisoma trivolvis* central nervous system and reverse transcribed to cDNA. Primers were designed from alignments of the prepropeptide coding sequences of mollusks, other invertebrates and vertebrates and were used in PCR reactions to amplify cDNA fragments from homologous reqions. 3' and 5' RACE were used to amplify both ends of the prepropeptide cDNAs. Full length *Helisoma* GnRH cDNA encodes a 109 amino acid precursor that contains an 11 amino acid mature hormone that is 91% identical to *Aplysia* GnRH. The whole precursor, however, is only 37% identical to the *Aplysia* prepropeptide. *Helisoma* APGWamide cDNA encodes a 213 amino acid precursor that contains

eight cleavable APGWG repeats from which the mature APGWamide may be derived enzymatically. The precursor is 55% identical to *Lymnaea stagnalis* APGWamide precursor which contains ten cleavable APGWG sequences. Four different cDNAs from *Helisoma* appear to code for SCP precursors, representing combinations of two separate insertion/deletions. The shortest form codes for a 112 amino acid precursor that contains two mature nonapeptide sequences that resemble *Lymnaea* SCP peptides (67 and 100% identity to *Lymnaea* SCP_A). The longer versions contain another possible mature nonapeptide with 33% homology to the other *Helisoma* peptides. These cDNAs will be used to make riboprobes to localize the peptide precursors to pond snail tissues and to synthesize peptides for activity in *Helisoma* and *Biomphalaria glabrata*, the intermediate host to the schistosomiasis parasite. These sequences also enhance our understanding of the families of neuromodulators that control motor behaviors in mollusks and other organisms.

Disclosures: N.W. Kleckner: None. **C. O'Leary:** None. **J. Bergeron:** None. **A. Hulse:** None. **M. Arsnow:** None. **D. Birkhead:** None. **G. Borland:** None. **P. Dixon:** None. **H. Fisher:** None. **C. Garvey:** None. **J. Meyo:** None. **V. Jarvis:** None. **A. Sridhar:** None. **J.D. Sato:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.08/ZZ12

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS081013

Title: Modulator induced changes in motor patterns are temperature compensated

Authors: *S. A. HADDAD, E. MARDER;
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Abstract: Central pattern generators (CPGs) are responsible for many life essential behaviors. The stomatogastric ganglion (STG) in the rock crab, Cancer borealis, is a well characterized CPG that controls digestion and filtering in the stomach. Here, we study phase relationships, between the subset of STG neurons that comprise the pyloric rhythm, in response to temperature change and modulatory perturbation. It is known that phase relationships in the pyloric network are maintained across an environmentally realistic temperature range. It is also known that the vast range of cellular processes (channel opening and closing, ligand-substrate binding, enzymatic reactions) have different Q10's (the Δ in rate of a process/ 10°C). Therefore, it is not obvious how the network compensates to maintain phase relationships given the plethora of

temperature variable cellular processes. To study this, each dissected stomatogastric nervous system (STNS) is subjected to three temperature ramp (11°C-31°C in 4° increments) protocols. In the first ramp, the STG receives modulatory input from descending neurons (front end-on). We find that the network frequency increases from ~ 1 hz at 11°C to ~3hz at 31°C . For the second ramp, the descending inputs are severed (decentralized). Under this condition, the frequency of the pyloric rhythm is significantly reduced and on occasion, the triphasic rhythm is lost. Network frequency increases from ~0.5 hz to ~1.5 hz from 11°C to 31°C, respectively. In the third temperature ramp, the preparation (still decentralized) is superfused with saline containing a modulatory substance, either 10-6 M proctolin or 10-5 M oxotremorine, a muscarinic cholinergic agonist. Both open the same modulatory inward conductance, IMI , and have differential densities of receptors depending on cell types. The frequency increase in the oxotremorine condition closely mimics the control condition, averaging ~1hz to ~4hz from 11°C to 31°C, respectively. Conversely, the frequency increase in the proctolin condition more closely mimics the decentralized condition, averaging ~0.5hz to ~1.75hz from 11°C to 31°C, respectively. Interestingly, we show that phase relationships are maintained in the network across the experimental range of temperatures and the two modulatory conditions. Although the neuromodulatory substances differentially effect the degree to which network frequency increases with temperature, in both cases, phase relationship are remarkably similar to the control (front end-on) condition.

Disclosures: S.A. Haddad: None. **E. Marder:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.09/ZZ13

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: ISF 1591/08 (ALT)

ISF 1930/08 (ALT)

ISF 491/12 (ALT)

ISF 685/01 (LA)

MDA (LA)

Title: Sacral muscarinic-acetylcholine receptors mediate the cholinergic modulation of locomotor-related motor output produced by sacrocaudal afferent stimulation in the neonatal rat spinal cord

Authors: L. ANGLISTER, E. FINKEL, A. ETLIN, M. CHERNIAK, Y. MOR, *A. LEV-TOV; Dept. Med. Neurobiol., IMRIC, Hebrew Univ. Sch. of Med., Jerusalem, Israel

Abstract: Synaptic excitation by sacrocaudal-afferent (SCA) input of sacral relay neurons projecting rostrally through the ventral white-matter funiculi (VF-neurons) is a potent activator of the hindlimb central pattern generators (CPGs) in the isolated rodent spinal cord. Wavelet analysis of the motor output produced in the lumbar spinal segments by SCA-stimulation following sacral application of the acetylcholinesterase blocker edrophonium (EDR) revealed ~10% decrease in the frequency (N-frequency) of the rhythm and >40% increase in its coherent cross power (N-power). The effects of EDR were reversed by sacral application of the muscarinic antagonist atropine or the M2-type receptor antagonist, methoctramine. Immunostaining of the sacral cord revealed that the majority of the VF- neurons express M2- and that some of them express M3-muscarinic acetylcholine-receptors. The sacral VF-neurons also exhibited acetylcholinesterase activity, ability to synthesize acetylcholine, and/or innervation by cholinergic synaptic inputs. Calcium imaging of sacral VF-neurons revealed modification of their activity in the presence of sacral-EDR during SCA-stimulation. We suggest that variations in the sacral level of acetylcholine modulate the SCA-induced locomotor rhythm via muscarinic receptor-dependent mechanisms, that the decreased N-frequency in the presence of sacral-EDR is accounted for by the reduced drive provided by sub-population of VF-neurons to the lumbar CPGs, and that the increased N-power reflects an enhanced activity of sub-population of VF-neurons that project directly and indirectly to lumbar motoneurons. The possible mechanism and implications of the involvement of sacral cholinergic-system in modulation of the locomotor-related motor output in the absence of descending supraspinal control will be discussed.

Disclosures: L. Anglister: None. **A. Lev-Tov:** None. **E. Finkel:** None. **A. Etlin:** None. **Y. Mor:** None. **M. Cherniak:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.10/ZZ14

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant R37-NS029436

Title: Circuit state-dependent responses to sensory feedback and hormonal modulation

Authors: *J. C. RODRIGUEZ, M. P. NUSBAUM;
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Abstract: We are addressing the hypothesis that distinct circuit states producing the same output pattern respond differently to the same input. We are testing this hypothesis using the crab (*Cancer borealis*) stomatogastric nervous system, where bath-applying CabPK peptide ($\geq 10^{-7}$ M) and stimulating the projection neuron MCN1 elicit the same gastric mill (chewing) motor pattern despite using different rhythm-generating mechanisms (Saideman et al, 2009 J Neurosci; Rodriguez et al, in prep). MCN1 does not contain CabPK and is silent during the CabPK-rhythm. The MCN1-rhythm response to sensory feedback and circulating hormones is known (DeLong & Nusbaum, 2010 J Neurosci). For example, the GPR sensory neuron relays muscle stretch information to the MCN1-gastric mill circuit, slowing the chewing rhythm by prolonging the retractor phase, while the hormone CCAP slows this rhythm by prolonging the protractor phase. We aim to determine the CabPK-chewing rhythm response to these same inputs, including the underlying mechanisms.
Here we show that the CabPK (10^{-6} M)-gastric mill rhythm exhibits the same response to GPR stimulation as the MCN1-rhythm, but is differentially sensitive to CCAP application. Specifically, GPR stimulation selectively prolonged the CabPK-retractor phase (Retraction: control, 9.9 ± 1.0 s; GPR, 24.6 ± 2.7 s, n=7, $p<0.05$; Protraction: n=7, p=0.1). This conserved GPR action, however, was mechanistically distinct. GPR alters the MCN1-rhythm by inhibiting the MCN1 axon terminals (DeLong et al, 2009 J Neurophysiol). In contrast, GPR appears to alter the CabPK-rhythm by inhibiting the rhythm generator neuron LG. For example, GPR stimulation during the LG burst (protractor phase) prematurely ends this burst prior to the return of activity in the retractor neurons that inhibit it (n=3), and focally applying the GPR cotransmitter (5HT) that inhibits LG also prolongs retraction (n=2). Thus, GPR uses distinct mechanisms to produce an invariant gastric mill rhythm response. With respect to CCAP modulation, whereas CCAP ($\leq 10^{-8}$ M) slows the MCN1-rhythm by prolonging protraction, the CabPK-rhythm is insensitive to its presence (Protraction: n=7, p=0.66; Retraction: n=7, p=0.11). Further, higher CCAP levels (10^{-6} M) increase the CabPK-rhythm speed by reducing retraction duration (Retraction: control, 11.4 ± 0.97 s; CabPK, 8.1 ± 0.76 s; n=4, $p<0.05$; Protraction: n=4, p=0.1), opposite to the CCAP action on the MCN1-rhythm. These results demonstrate that different network states that produce the same steady-state output can be differentially sensitive to a shared input, while maintaining responsiveness to other ones, albeit via distinct synaptic mechanisms.

Disclosures: J.C. Rodriguez: None. **M.P. Nusbaum:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.11/ZZ15

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR

Title: Dopaminergic contribution to locomotion in the neonatal and adult mouse

Authors: *S. A. SHARPLES[1], J. HUMPHREYS[2], S. A. DHOOPAR[2], N. DELALOYE[2], A. KRAJACIC[2], S. NAKANISHI[2], P. J. WHELAN[2];
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Abstract: Central pattern generators (CPGs) are networks of neurons that are capable of generating rhythmic outputs in the absence of phasic input. CPGs located within the spinal cord are responsible for producing the rhythmic patterns that produce locomotion and are modulated by descending monoaminergic centers from the brainstem. In particular, our lab has established the ability of dopamine (DA) to modulate the locomotor CPG in the neonatal mouse (P0-3) by reducing the frequency and increasing the regularity of the locomotor rhythm. What is still unclear are the mechanisms that mediate this effect. We first tested the dose of DA sufficient to elicit a reduction in locomotor frequency and increase in power and found that doses as low as 10μM were able to modulate the rhythm. We hypothesized that the reduction in frequency of the rhythm in the neonate is mediated by D2-like receptors. In contrast to our hypothesis, administration of the D2 receptor antagonist, L741,626 (6-12μM), did not affect the DA-induced (35μM) modulation of locomotor rhythm, however, sulpiride (20μM), a more general D2/D3 antagonist offset DA effects, increasing locomotor frequency and decreasing power. We suggest that lower concentrations of DA compared to those traditionally used (50μM) are sufficient to evoke regular and long-lasting bouts of rhythmicity. DA appears to be reducing frequency by acting mainly on D3 receptors. In addition, we demonstrate the ability of DA to increase the frequency of locomotor activity in the adult decerebrate mouse preparation. This points to the possibility of a change in DA receptor expression in the spinal cord over development, however further experiments will be required to clarify these differential effects.

Disclosures: S.A. Sharples: None. **J. Humphreys:** None. **S.A. Dhoopar:** None. **N. Delaloye:** None. **A. Krajacic:** None. **S. Nakanishi:** None. **P.J. Whelan:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.12/ZZ16

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF Research Grants 0135162 and 0641326

ANR grant NT05-1_41718

Title: Opposing effects of serotonin at different sites within the same neuron

Authors: J. BACQUE-CAZENAVE[1], F. A. ISSA[2], D. CATTAERT[3], *D. H. EDWARDS[2]; [1]Biol., Univ. of Bordeaux 1, Bordeaux, France; [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [3]Ctr. de Neurosciences Integratives et Cognitives, Univ. of Bordeaux 1 and Bordeaux 2, Bordeaux, France

Abstract: The different modulatory effects of serotonin are often apparent in different neurons, but they can also appear in a single neuron when different parts of the cell are exposed to the modulator. We have observed this in leg depressor (Dep) motor neurons (MNs) of crayfish, where serotonin focally applied to the cell's dendritic arbor had excitatory effects, whereas the cell was inhibited when serotonin was applied to the cell's initial axon segment at the nerve root. Both inhibitory and excitatory serotonergic effects persisted in low calcium saline when polysynaptic reflexes were blocked, suggesting that serotonergic effects are direct. Moreover, numerous close appositions between 5-HT immunoreactive processes and a labeled Dep MN (injected with dextran rhodamine) were found in the region of the MN's initial segment, whereas almost no close apposition sites were found in the Dep MN dendritic arbor. We suggest that the 5-HT controls the level of excitability of postural network MNs by two mechanisms acting at two different sites: inhibitory responses (consistent with an action involving the opening of K+ channels) occur in the initial segment region and may involve classical synaptic transmission, while depolarizing responses (consistent with an action involving closing of K+ channels) occur on MN branches via apparently paracrine effects. Finally, we found that intracellular stimulation of an identified serotonergic interneuron in the first abdominal ganglion produced either excitatory or inhibitory effects on Dep motorneurons in different preparations. These A1 5-HT neurons project into the thoracic ganglion and have axonal arbors in both the central dendritic region of the Dep motor neurons and the initial axon segment in the motor nerve root.

Disclosures: J. Bacque-Cazenave: None. **F.A. Issa:** None. **D. Cattaert:** None. **D.H. Edwards:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.13/ZZ17

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG STE 937/8-1

Title: The Huber-Braun model as a candidate for modeling central pattern generators

Authors: *Q. SKILLING[1], E. ROSA, JR.[2], W. STEIN[3];
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Abstract: Neuronal oscillators such as central pattern generators (CPGs) are ubiquitous throughout the nervous system and often drive vital behaviors such as breathing, swallowing, and chewing. While many modulatory influences have been shown to affect CPG activity, motor patterns must also be robust against perturbations and function over a broad range of frequencies and modulatory conditions.

We are studying pattern generation and the stability of oscillatory activity using computer modeling of the pyloric (filtering of food) and gastric mill (chewing) CPG circuits in the crustacean stomatogastric nervous system. The type of model used for CPG circuits varies greatly, but in general, simplified integrate-and-fire neurons ignore many of the dynamics of the biological neurons, while detailed modeling often requires extensive knowledge about intrinsic neuronal properties and represent only a snapshot of a particular set of neuronal responses. They can often only be studied numerically. Yet, to characterize the dynamics of the system, analytically tractable models are preferable.

The fast pyloric rhythm is mainly driven by subthreshold oscillatory ionic currents and graded synaptic release, and can be challenged with temperature perturbations to achieve a frequency range of 0.5 - 3 Hz before it crashes. A two-dimensional nonspiking Morris-Lecar model is sufficient to explain network stability in this frequency range [Rinberg, Taylor, Marder, PLOS Comput Biol 2013]. The slow gastric mill rhythm (GMR) operates in a different frequency domain (0.2 - 0.05 Hz), but also includes neurons that support fast pyloric-timed influences. It is mainly network driven, requires spiking and intrinsic oscillatory properties, and can be entrained to different frequencies by synaptic input. We are testing the four-dimensional Huber-Braun (HB) model, which was developed to account for temperature dependent behaviors, as a candidate for capturing the rich dynamics of this CPG circuit. The HB model uses biophysically realistic membrane currents that bring about subthreshold oscillations as well as fast spiking and create a rich repertoire of spike and burst dynamics. Here, we are testing the oscillatory properties of the HB model by determining resonance and spike entrainment properties, as well as changes in firing regime.

Our preliminary data shows that the burst and spike activity of the HB model can be entrained in a frequency range that roughly corresponds to that of the GMR, indicating that the model could

operate in a CPG with these frequencies. Currently, we are determining the resonance properties of the model and its capabilities for accurately representing a CPG circuit.

Disclosures: Q. Skilling: None. **E. Rosa, Jr.:** None. **W. Stein:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.14/ZZ18

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Capacity building award in Integrative Mammalian Biology

Medical Research Scotland

Title: Long term effects of Fentanyl on postnatal breathing patterns

Authors: *L. C. MCKAY, A. KENNEDY;
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Abstract: The mammalian respiratory system is immature at birth. In mice, this immaturity is characterized by a fragile and highly variable breathing pattern during postnatal days 1-3 (P1-3). Around P4, the respiratory
system undergoes a step in maturity, after which breathing is less variable and has a higher frequency. The neural mechanisms underlying this maturity step are unknown. The preBÖtzinger Complex (preBÖtC) and Retrotrapezoid nucleus/parafacial respiratory group play a critical role in generating respiratory rhythm but little is known of their interaction during development and early postnatal life when the respiratory system is fragile. Fentanyl, a μ-opioid receptor agonist was used to pharmacologically manipulate the opiate sensitive preBÖtC during early postnatal life and to investigate the long term effects on breathing of being exposed to opiates during this critical period of postnatal maturation. Neonatal mice were exposed to fentanyl (0.08mg/kg i.p daily), or saline as a control, from P1-5 (n=16) or P9-13 (n=16).
Mice were continuously monitored post injection and breathing recorded by closed plethysmography at regular intervals from 5 minutes to 2 hours post injection. Fentanyl had a modest effect on breathing at all postnatal days by increasing variability, decreasing frequency (250±20 *vs* 150±30 breaths per minute) and increasing the number of apneas (2±1 vs 5±2 per minute), compared to saline-exposed mice. At 6 weeks of age, all saline and fentanyl exposed mice were exposed to a further dose of fentanyl (0.04 - 1.0mg/kg ip) and monitored as above. Respiratory frequency was significantly decreased (190±10 *vs*120±15 bpm, $p<0.05$) in all mice

previously exposed to saline as neonates (P1-P5 and P9-13); however, in mice previously exposed to fentanyl as neonates (P1-P5 and P9-13), further fentanyl exposure in adulthood had no effect on respiratory frequency (180±8 *vs* 170±10 bpm).
Tidal volume increased slightly in all mice post fentanyl regardless of whether they had previously been exposed to fentanyl or saline. These data suggest that the respiratory system in younger animals is less susceptible to fentanyl compared to adults and pre exposure to fentanyl during early postnatal maturation results in a long term desensitization to further fentanyl insults.

Disclosures: L.C. Mckay: None. **A. Kennedy:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.15/ZZ19

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Craig Neilsen Foundation

Title: Do sympathetic preganglionic neurons modulate locomotion by intraspinal activity-dependent release of nitric oxide?

Authors: *P. LOSEY, S. HOCHMAN;
Physiol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Though known to receive common input from several descending pathways involved in behavioral drives, intraspinal interactions between activity in sympathetic preganglionic neurons (SPNs) and motor neurons are not thought to occur. Interestingly, only SPNs express nitric oxide synthase (e.g. Dun et al 1992), and intraspinal nitric oxide (NO) complexly modulates vertebrate motor output including locomotor activity (e.g. Kyriakatos et al 2009). Here we sought to determine whether SPN neuronal activity derived NO can contribute to the modulation of locomotor like activity (LLA) in the isolated mammalian spinal cord.
We undertook studies in the isolated neonatal rat spinal cord, some with intact sympathetic chain to independently record SPN activity. We stimulated caudal lumbar or sacral dorsal roots to evoke LLA recorded predominantly in L1 and L2 ventral roots (VRs) known to also contain SPN efferent axons. We observed that during afferent stimulation-induced LLA, SPNs are also recruited to spike. When sympathetic bursting activity continued after stimulation epochs, these bursts were synchronous with those seen in VRs bilaterally. This indicates that some of the rhythmic activity observed in L1-2 VRs reflects firing in SPNs.
We wondered whether spiking activity in SPNs could modulate ongoing LLA via a nitrergic

mechanism since Wu and Dun (1995) showed that high frequency activity in SPNs was sufficient to induce facilitation of excitatory transmission via Ca2+ dependent release of NO. Repetitive trains of stimuli were delivered to VRs at intervening periods between bouts of stimulation evoked LLA (3x10 epochs at 50 Hz) or concomitant with DR stimulation induced LLA (4x5 pulses at 30-50 Hz; n=4). In all cases the amplitude of motor output was facilitated on the contralateral side while ipsilateral motor activity was depressed. Both facilitatory and depressant actions appeared long-lasting (> 1 hour). To associate these actions with NO we tested the effects of the NO donor DEANO (50 μM) on LLA. In the presence of DEANO, motor burst amplitude changes were complex and included amplitude increases and decreases with amplitude increases more consistently observed following washout across all VRs (n=4). These preliminary results suggest spiking activity in SPNs may utilize intraspinal nitrergic volume transmission to directly modulate motor function.

Disclosures: P. Losey: None. **S. Hochman:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.16/ZZ20

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF Grant IOS-1121973

NIH Grant 5P20RR016463-12 from NCRR

NIH Grant 8P20GM103423-12 from GMS

Title: Modulation of the lobster cardiac neuromuscular system: Roles and mechanisms of two related neuropeptides

Authors: *P. S. DICKINSON, A. M. CALKINS, J. S. STEVENS;
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Abstract: Neural networks controlling rhythmic motor behaviors can be modulated on a number of different levels, including the pattern generator itself and the response of the muscle to a given pattern of motor output. One family of modulatory neuropeptides in the American lobster, *Homarus americanus*, is the FMRFamide-related peptides, at least 18 of which have been identified. We examined the role of two peptides from this family, GYSDRNFLRFamide (GYS) and SGRNFLRFamide (SGRN), in modulating the neurogenic lobster heartbeat, which is

controlled by the cardiac ganglion (CG) located within the lumen of the heart. When perfused through an isolated whole heart at concentrations ranging from 10^{-11} to 10^{-8} M, both GYS and SGRN elicited large and similar increases in contraction amplitude. Amplitude increased by over 100% at the higher concentrations, with smaller increases at lower peptide concentrations. In contrast to the consistent effects of GYS and SGRN on contraction amplitude across concentrations, the effects of the two peptides on contraction frequency were similar at low concentrations, but differed at higher concentrations. Thus, at low concentrations, both peptides elicited increases in frequency, but at high concentrations (e.g., 10^{-8} M), SGRN continued to significantly increase contraction frequency, while GYS significantly decreased frequency. Moreover, when we recorded CG motor output in semi-intact hearts, GYS significantly increased both contraction duration and motor neuron burst duration at higher peptide concentrations, while SGRN did not elicit significant changes in either of these parameters. To determine the mechanisms through which these two peptides exert their effects, we examined the effects of the peptides on the periphery (neuromuscular junction and cardiac muscle) as well as their effects on the motor neuronal output in the isolated CG. We thus removed the CG and stimulated the motor nerve with constant bursts of stimuli, designed to mimic the CG output. When neural input was held constant, both GYS and SGRN elicited similar increases in contraction amplitude, indicating that the two peptides similarly modulate the muscle and/or the neuromuscular junction. When applied to the isolated CG, both peptides significantly increased burst duration and the number of action potentials per burst. However, GYS did not alter cycle frequency, whereas SGRN significantly decreased burst frequency, suggesting that the differences in responses of the whole heart to the peptides at higher concentrations are due largely to the differential modulatory effects of GYS and SGRN on the CG.

Disclosures: P.S. Dickinson: None. **A.M. Calkins:** None. **J.S. Stevens:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.17/ZZ21

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH NS066587

NS070583

Title: Functional implications of distinct transmitter phenotypes in higher-order interneurons

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Abstract: The specific complement of neurotransmitters, especially, peptide modulators, that are expressed in a particular interneuron may help determine its functional identify, but the underlying mechanisms remain poorly understood. We examine this issue in three electrically-coupled higher-order interneurons, cerebral-buccal interneuron-2 (CBI-2), CBI-11 and CBI-3, that activate/modulate feeding motor programs with biphasic protraction-retraction sequences in the mollusc *Aplysia californica*. Although electrical coupling between the interneurons suggests that they are functionally related, only CBI-2 and CBI-11 express the peptide FCAP that promotes motor program generation. This is consistent with the fact that CBI-2 and CBI-11, but not CBI-3, can drive motor programs. Previous work showed that CBI-2 also contains peptide CP2 that enhances motor program generation. We show, however, that CBI-11 is not immunoreactive to CP2. Consistent with this, CBI-11 requires a higher firing frequency than CBI-2 to reliably drive motor programs. In addition, the latency to protraction initiation is longer when stimulating CBI-11 than CBI-2. Interestingly, CBI-2 pre-stimulation shortens the latency of programs elicited by CBI-11, which may be accounted for by the actions of CP2 present in CBI-2. On the other hand, we found that CBI-11 also modulates CBI-2-elicited programs, making them more ingestive and extending the protraction duration. These CBI-11 effects can be accounted for, at least partially, by CBI-11's abilities to enhance the activity of the ingestion-promoting interneuron B40 and to inhibit the retraction interneuron B64. Thus, distinct transmitter phenotypes of higher-order interneurons provide one effective means of defining their functional classes.

Disclosures: J. Wu: None. **M. Siniscalchi:** None. **N. Wang:** None. **J. Gu:** None. **F.S. Vilm:** None. **E.C. Cropper:** None. **K.R. Weiss:** None. **J. Jing:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.18/ZZ22

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIMH Grant 64711

Title: Distinct regulatory mechanisms of ionic conductances in a central pattern generating network

Authors: *D. SALLOUM, J. GOLOWASCH;
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Abstract: Neurons express variable levels of ionic conductances but the sources of variability are unknown. Short-term regulation of ionic conductances by activity has been shown in the inferior cardiac (IC) neuron (Golowasch et al., 1999) and in cultured neurons (Haedo & Golowasch, 2006) of the pyloric network of the stomatogastric ganglion (STG) in the crab Cancer *borealis.* On the other hand, in pyloric dilator (PD) neurons, several ionic conductances have been shown to be regulated over long-term by neuromodulatory input. Khorkova & Golowasch (2007) showed that removal of neuromodulatory input to the pyloric network induces changes in ionic conductances of PD neurons over several days. This contrasts with the acute activation of a single target current (I_{MI}) by some of these neuromodulators. To maintain these conductances within physiological limits, neurons may possess mechanisms that link the effects of activity and neuromodulation. We hypothesized that there is an interaction between activity-dependence and neuromodulatory control of the ionic conductances in pyloric neurons. We tested the effects of activity in control preparations devoid of neuromodulatory input (decentralized) and in preparations in which neuromodulators were reintroduced exogenously. We predicted that neuromodulators would regulate activity-dependent changes of the K^+ currents in PD neurons. We tested activity-dependence by stimulating PD cells with depolarizing voltage steps at 1Hz in voltage clamp. We observed that PD neurons exhibit time-dependent changes in the high-threshold (I_{HTK}) and transient (I_A) potassium currents, with I_{HTK} conductances decreasing and I_A conductances increasing over time, but that there was no effect of induced activity. Applying a 'cocktail' of four neuromodulators has a significant effect on the K^+ currents in comparison to control conditions, independent of activity: in the case of I_{HTK} the time-dependent decay of the current is significantly larger in magnitude than in control preparations. In the case of I_A neuromodulators significantly inhibit the increase of current conductance seen in control preparations. The leak conductance does not change significantly in response to either of these treatments.
In summary, IC alters its current conductances in an activity dependent manner (Golowasch et al. 1999), while the PD neurons do not. Instead, PD neurons modify their ionic conductances depending on the neuromodulatory input they receive. We conclude that different cell types within the pyloric network in the crab STG have distinct mechanisms of regulating their ionic conductance levels depending on activity and neuromodulatory input.

Disclosures: D. Salloum: None. **J. Golowasch:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.19/ZZ23

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DoD CDMRP SC090555

Title: The effects of neuromodulation on output and synchrony in electrically coupled motor neurons with variable intrinsic conductances in the crab cardiac ganglion

Authors: *B. J. LANE[1], J. L. RANSDELL[2], P. SAMARTH[3], S. S. NAIR[3], D. J. SCHULZ[2];
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Abstract: The capability of neurons and networks to generate reliable outputs as well as to maintain appropriate phase is a critical aspect of nervous system organization. In addition, many networks require short term adaptability influenced by neuromodulation. One such network which clearly displays the convergence of these demands is the cardiac ganglion (CG) of the crab *C. borealis*. This small central pattern generator network consists of 4 small pacemaker cells that give excitatory input to 5 bursting large cell motor neurons (LCs) which are responsible for the monophasic contraction of the crab's single-chambered heart. Previous work has shown that individual LCs show substantial variability in their underlying voltage-gated conductance magnitudes, including two potassium currents, I_A and I_{HTK}. These cells are able to maintain consistent output in part by differentially balancing conductance magnitudes, as revealed when pharmacological blockade of I_A or I_{HTK} caused disparate outputs across network LCs. If multiple neurons within the same network achieve synchronized output despite variable underlying conductances, then what are the implications for neuromodulation, known to target subsets of ionic conductances, for neuron output and synchrony in this model system? Here we identify serotonin as a neuromodulator with direct effects on LC motor neurons, and further show that serotonin increases I_A magnitude but has no significant effect on I_{HTK} magnitude. The activation voltages of both I_A and I_{HTK} remain unaffected. We then test the effects of these changes in I_A on changes in LC neuron output both at the level of individual isolated neurons as well as within the intact network. By distinguishing the effects of neuromodulation on individual cells with variable underlying conductances from effects on the entire network output, we can begin to determine whether modulation itself is capable of resulting in conserved output, or whether higher level network properties (such as electrical coupling) constrain variability in output to ensure robust activity in this network which is critical for survival.

Disclosures: B.J. Lane: None. **J.L. Ransdell:** None. **P. Samarth:** None. **S.S. Nair:** None. **D.J. Schulz:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.20/ZZ24

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Robust cardiac ganglion network output with large cells having variable conductances

Authors: *P. S. SAMARTH[1], J. L. RANSDELL[2], D. J. SCHULZ[2], S. S. NAIR[1];
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Abstract: The crustacean cardiac ganglion (CG) network in Cancer borealis consists of 9 cells, 5 'large cell' motor neurons (LCs) and 4 small endogenous pacemaker cells (SCs). The CG network coordinates the rhythmic bursting of the heart muscle contractions to control the circulation of blood. We developed a biophysical model for the LCs using data from currents measured in our Lab. Our data suggests that although the five LCs burst synchronously, their underlying ionic conductances vary considerably, even within the same animal. We created a nominal model using the biological conductance data and performed statistical rejection sampling to determine parameter sets that preserve LC output characteristics. To be included in the model cell data set, a given LC model had to have passive properties within biological bounds and then have appropriate output responses to multiple current injection protocols that are consistent with experimental traces.Parameter sets obtained in this fashion were mined to predict relationships that might exist between currents in native LCs. We then use multiple LC models with variable underlying conductances to study the role of electrical coupling in synchronizing LC responses in multiple model cardiac ganglion networks.

Disclosures: P.S. Samarth: None. **J.L. Ransdell:** None. **D.J. Schulz:** None. **S.S. Nair:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.21/ZZ25

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Graduate School Fellowship

Dr. Linton E. Grinter College of Medicine Graduate School Fellowship

National Institute of Neurological Disorders and Stroke Grant NS058825

Title: Cell-type specific neuropeptide receptor transcript levels are correlated with physiological response threshold

Authors: *V. J. GARCIA[1], S. TEMPORAL[2], D. J. SCHULZ[3], D. BUCHER[4];
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Abstract: The crustacean stomatogastric ganglion (STG) is modulated by a multitude of substances, mostly neuropeptides. Apart from some effects on synapses, most peptides converge onto the same voltage-gated current (IMI). Peptide-specific effects on circuit activity are thought to arise mainly from modulation of different subsets of neurons by different peptides. However, in the absence of identified crustacean neuropeptide receptors, the role quantitative differences in receptor expression across cell types could play in circuit modulation is unclear. Furthermore, as second messenger signaling and downstream target activation could differ quantitatively, it is unknown how receptor expression levels correlate with the magnitude or sensitivity of current responses. Here we describe the complete coding sequence of the putative crustacean cardioactive peptide (CCAP) receptor in the crab Cancer borealis. We show neuron-specific differences in mRNA expression level correlated with differences in threshold of receptor-mediated IMI activation in STG neurons.
The CbCCAPr is a G protein-coupled receptor (GPCR), homologous to various insect cardio-acceleratory peptide receptors, as well as mammalian vasopressin and oxytocin receptors within the rhodopsin-like GPCR superfamily. PCR employing gene-specific primers reveals the presence of CbCCAPr transcripts in a variety of nervous tissues, including the STG. We show that CbCCAPr transcripts are present in only a subset of neuron types in the STG, and, with one exception, only in neuron types that show IMI activation in response to CCAP application. The exception is one neuron that does not show IMI activation but displays a CCAP mediated increase in responses to synaptic input.
Quantitative RT-PCR reveals variability in transcripts across 3 different CbCCAPr-expressing neurons, but only moderate variability within each cell type across individuals. The IC neuron shows moderate expression, while the LP and LG neurons show 4-5 times higher expression. Measurements of IMI responses show inconsistent peak current values. However, expression levels correlate well with concentration dependence. LP and LG show an activation threshold and EC50 about 1 order of magnitude lower than IC. Occlusion experiments with other peptides reveal that in LP and LG, micromolar CCAP is sufficient to fully activate IMI. In contrast, CCAP does not occlude IMI responses to other peptides in IC. These findings suggest that there can be large quantitative differences in neuropeptide responses in different cell types, with potentially important consequences for circuit modulation.

Disclosures: V.J. Garcia: None. **S. Temporal:** None. **D.J. Schulz:** None. **D. Bucher:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.22/ZZ26

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DoD CDMRP hypothesis and exploration award #SC090555

GAANN Fellowship

Gus T. Ridgel Fellowship

Title: Removal of neuromodulation increases receptor transcript abundance and subsequent response to a peptide neuromodulator in lp neurons of the crab stomatogastric ganglion

Authors: *K. LETT[1], V. J. GARCIA[2], D. BUCHER[2], D. J. SCHULZ[1];
[1]Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO; [2]Whitney Lab. and Neurosci. Dept., Univ. of Florida- St. Augustine, St. Augustine, FL

Abstract: Neuromodulation is essential for regulating and initiating the activity and the resulting appropriate physiological output of central pattern generating (CPG) networks. Elimination of a neuromodulatory source can result in a change in sensitivity to restored modulatory input. Crustacean Cardioactive Peptide (*Cb*CCAP) has been shown to be released solely hormonally and is able to alter the pyloric network output of *C. borealis* stomatogastric ganglion (STG) by increasing the activity of the lateral pyloric neuron (LP). We have shown previously that the pyloric network exhibits differential sensitivity to restored modulation when deafferented. Similar findings were observed in other models where sensitivity to neuromodulatory input is increased after injury. We hypothesize that removing hormonal and descending modulatory input to the LP neuron will alter its excitability and sensitivity to CCAP. We further hypothesize that these changes may be due to modification of *Cb*CCAP receptor expression which in turn affects inward currents that contribute to excitability in LP. We investigated this by quantifying neuromodulator receptor mRNA in individual LP neurons using quantitative RT-PCR and measuring neuronal excitability in the presence of different concentrations of *Cb*CCAP using current clamp. Preparations were subdivided into 3 groups to address the hypothesis; control, decentralized for 24 hours in culture, and decentralized and CCAP incubated for 24 hours. Overall we observed a concomitant increase in *Cb*CCAPr transcript and CCAP-induced excitability in the LP neuron when neuromodulatory input was removed from the pyloric network. Incubation of the ganglion with the peptide for 24 hours prevented this increase in the *Cb*CCAPr transcript and the change in excitability in LP despite the lack of descending input from the commissural ganglia. Our findings suggest that there is a compensatory change in the

excitability of individual neurons of a deafferented motor network which is likely attributed to modification of neuromodulator receptor mRNA expression and intrinsic excitability.

Disclosures: K. Lett: None. **V.J. Garcia:** None. **D.J. Schulz:** None. **D. Bucher:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.23/AAA1

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant R01-NS65054

Title: The integrities of spontaneous and hunting locomotor repertoires of zebrafish larvae are dissociably dependent on the conserved dopaminergic diencephalospinal tract

Authors: *A. M. LAMBERT, M. A. MASINO;
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Abstract: Zebrafish larvae employ a hunting repertoire of short, slow, and fine orienting and advancing locomotor maneuvers during prey-tracking of paramecia. Most of this hunting repertoire resembles their mature spontaneous locomotor repertoire at 4-7 days-old, and we have shown that the ontogeny of the latter conspicuously correlates with the ontogeny of prey capture performance. Interestingly, we have found that chemogenetic ablation of the dopaminergic diencephalospinal tract (DDT) or antagonism of D4 receptors, through which the DDT putatively signals, relegates 5-7 day-old larvae to an immature spontaneous locomotor repertoire of long, fast, erratic swimming episodes and compromises prey capture performance. This suggests that the integrity of the spontaneous locomotor repertoire is predictive of prey capture performance and that the DDT plays a role in both phenomena. Here, we investigated the cause of the compromised prey capture performance of DDT-perturbed larvae by assessing paramecia-induced changes in locomotor activity and, moreover, the kinematics of prey encounters and the integrity of the hunting repertoire.
Addition of paramecia to the arena reversibly increased the total distance travelled by both controls and DDT-perturbed larvae, suggesting that both groups were similarly engaged with paramecia. During prey encounters, DDT-perturbed larvae detected and pursued paramecia, similar to controls, by initiating and maintaining ocular convergence while attempting hunting locomotor elements, including hunting-specific j-turns. Controls employed short, slow and orienting locomotor elements that effectively reduced their distance and angular heading to paramecia before attempting prey capture. Surprisingly and, unlike their spontaneous activity,

DDT-perturbed larvae did not exhibit any long, fast, erratic swimming while engaged in prey encounters. In contrast, they attempted the finely controlled locomotor elements observed in controls, but were unsuccessful in reducing their distance or angular heading to paramecia due to executing bradykinesia-like swims and turns that often resulted in negligible displacement and shallow orienting.
Collectively, these results suggest that DDT-perturbed larvae have functional vision and visuomotor initiation, respectively, to detect paramecia and attempt hunting locomotor elements toward them. However, while ocular convergence induces a predatory mode of behavior in DDT-perturbed larvae that surprisingly and powerfully overrides their spontaneous bias for erratic swimming, it exploits their lack of fine motor control which results in compromised prey capture performance.

Disclosures: A.M. Lambert: None. **M.A. Masino:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.24/AAA2

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Swedish Research Council (VR-M 3026).

Title: Substance P depolarizes lamprey spinal cord neurons by inhibiting background K+ channels

Authors: *C. T. PEREZ, R. H. HILL, S. GRILLNER;
Karolinska Inst., Stockholm, Sweden

Abstract: Substance P accelerates the burst frequency of fictive locomotion, which is known to be endogenously released in the spinal cord of the adult lamprey. This increase in burst frequency is achieved by multiple effects on interneurons and motoneurons, of which attenuation of calcium currents, potentiation of NMDA currents and reduction of the reciprocal inhibition have been studied. Membrane depolarization combined with a conductance decrease during substance P application has been reported however, the underlying mechanism has not been resolved. This study focuses on the possible effects of substance P on background K+ channels as the main source for this depolarization. Hyperpolarizing steps induced inward currents during whole-cell voltage clamp that were reduced by substance P. We further test for involvement of a two-pore domain channel being modulated by varying the extracellular pH. Lower pH values reduced the outward currents and increased input resistance. Anandamide a selective blocker of

the two-pore, TASK-1 subtype K+ channel was also tested and it reduced the current during voltage steps and prevented substance P effect on membrane properties. Inhibition of the background K+ channels induces depolarization, which is likely to contribute to the frequency increase observed with substance P during fictive locomotion.

Disclosures: C.T. Perez: None. **R.H. Hill:** None. **S. Grillner:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.25/AAA3

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: PhD Fellowship from the AFM

Title: Longitudinal analysis of the cholinergic neuromodulatory system in the spinal lumbar enlargement of SOD1 mutant mice

Authors: L. MILAN, G. COURTAND, L. CARDOIT, F. MASMEJEAN, M. GARRET, *S. S. BERTRAND;
INCIA CNRS UMR5287, Bordeaux, France

Abstract: Spinal cholinergic interneurons connect alpha-motoneurons through large “en passant” synapses associated with subsynaptic cisterns in the postsynaptic membrane, the C-boutons. These synapses have been demonstrated to be associated with the postsynaptic muscarinic type 2 (M2) receptors. Recent studies have reported divergent results about morphological reorganization of the C-terminals in a mouse model of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by the progressive loss of cortical and spinal motor neurons. To further extent these studies, we performed a longitudinal analysis of the number and morphological parameters of C-boutons at 6 different developmental stages (1 day postnatal: P1, P10, P21, P40, P75, P100) on lumbar motoneurones in SOD1 mice, an animal model of ALS, and age-matched control littermates. Interestingly, our result show that in early developpemental stages (P10-P21), the number of choline acetyltransferase positive (ChAT+) C-boutons was decreased by 50% in SOD1 mice compared to control animals. This discrepancy vanishes at P75 and is reversed at P100 with a higher mean number of C-boutons per motoneurons in SOD1 mice. The area of C-boutons as well as their distribution across the soma and proximal dendrites of motoneurones also exhibit shrinking differences between SOD1 and control mice. This analysis was completed by a western blot quantification of M2 receptors in ventral lumbar spinal cord and an immunohistochemical study of the expression of M2 receptors

into motoneuron membrane. A significant positive correlation was computed between M2 receptor expression and the presence of a C-terminal regardless of the animal age and genotype. The correlation powers were however significantly different between SOD1 and control animals. To investigate the physiological impact of C-boutons number and size reduction, we performed patch-clamp recordings from motoneurons in lumbar spinal cord slices. The sensitivity of SOD1 and control motoneurons to the wide range muscarinic agonist, oxotremorine, was compared in SOD1 and control P10 mice. The efficacy of commissural electrical stimulations in inducing sustained muscarinic responses in motoneurones was also tested.
Finally, a stereological method was used to count the cholinergic interneurones that originate the C-boutons in both SOD1 and age-matched control mice.
Altogether these data show that as early as the second postnatal week, the cholinergic synapses and their associated receptors are permanently reconfigured in SOD1 mutant mice to maintain an appropriate level of cholinergic control onto motoneurons.

Disclosures: L. Milan: None. **S.S. Bertrand:** None. **G. Courtand:** None. **L. Cardoit:** None. **F. Masmejean:** None. **M. Garret:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.26/AAA4

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG grant STE 937/8-1

German Academic Exchange Service

Illinois State University Startup Grant

Central Illinois Neuroscience Foundation

Title: Modulation of axonal spike initiation in a sensory neuron by descending modulatory projection neurons

Authors: *C. STAEDELE[1,2], W. STEIN[1];
[1]Sch. of Biol. Sci., Illinois State Univ., Normal, IL; [2]Inst. of Neurobio., Ulm Univ., Ulm, Germany

Abstract: Sensory inputs play a pivotal role for the execution of behavior and decision making by selecting appropriate motor output patterns. Yet, sensory information is state- and history-

dependent and can be modulated, resulting in sensory ambiguity such that identical stimuli elicit distinct sensory outputs. Generally, it is assumed that either sensory spike activity or the sensory influence on postsynaptic targets is modulated. Little is known about the modulation of spike activity in the sensory axon, despite the fact that spike propagation in many axons is affected by neuromodulators. Even less is known about the origin of axonal modulation and its functional relevance.

We are studying the modulation of the anterior gastric receptor (AGR, Smarandache & Stein, J Exp Biol, 2007), a muscle tendon organ in the stomatogastric nervous system of the crab, *Cancer borealis*. AGR's soma is located in the stomatogastric ganglion (STG) and protrudes one axon to the periphery and a second, central axon to its postsynaptic targets, modulatory projection neurons in the commissural ganglia. While each axon possesses a spike-initiation zone, the one in the central axon dominates AGR's spontaneous firing. This tonic spike activity (2-5 Hz) is functionally relevant and even small frequency changes cause prominent changes in the motor response (Daur et al, Europ J Neurosci , 2009).

Here we show for the first time that the axonal spike initiation zone is influenced by the actions of descending modulatory projection neurons. We found that the instantaneous firing frequency of AGR changed rhythmically (oscillations) when a gastric mill rhythm (GMR, the chewing motor pattern generated by neurons in the STG) is present. AGR firing frequency changed by about 35% during spontaneously occurring and sensory-induced motor patterns (N=33). Oscillations were timed by the GMR, with higher firing frequencies during the activity phases of the LG and GM motor neurons.

Using intracellular recordings from descending projection neurons, we found that the presence of the gastric mill rhythm was not sufficient to elicit the oscillations, but that they rather depended on the oscillatory activity of the projection neurons. In particular, the projection neurons MCN5 and CPN2 modulated AGR firing. AGR's central spike initiation zone is located close to the anterior neuropil of the STG, and correspondingly we found that AGR possesses 1 to 3 neurites in this region (average 1.85± 0.89, N=13).

Our results thus indicate that AGR's axonal spike initiation zone is synaptically influenced by the modulatory projection neurons. Currently, we are testing whether this influence is caused by chemical or electrical coupling.

Disclosures: C. Staedele: None. **W. Stein:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.27/AAA5

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG STE 973/8-1 (WS)

Illinois State University Startup Grant (WS)

Central Illinois Neuroscience Foundation (WS)

DAAD PROMOS Scholarship (LYB)

Title: Modulation of the voltage dependence of an electrical coupling by a hyperpolarization-activated inward current

Authors: *L. Y. BRAUN[1,2], A. M. YARGER[2], W. STEIN[2];
[1]Inst. of Neurobio., Ulm Univ., Ulm, Germany; [2]Sch. of Biol. Sci., Illinois State Univ., Normal, IL

Abstract: Electrical synapses play an important role in information propagation and processing in the nervous system by facilitating synchronization between coupled neurons. However, electrical coupling can be too weak to cause significant synchronization and little is known about its function in this case, but current flow across the gap can be modulated either directly, by affecting the gating of the gap junction, or indirectly via voltage-dependent membrane properties. (Curti & Pereda, J Neurosci 24, 2004; Herve & Derangeon, Cell Tissue Res 352, 2013). While the mechanisms of direct modulation are well understood, the interactions between electrical coupling and local voltage-gated ion channels and the effects of the modulation of the electrical coupling on the coupled neurons are often unclear.

We study electrical synapses in the stomatogastric nervous system of the crab, Cancer borealis, a well-characterized motor system that controls the rhythmic movements of the foregut. In this system, the modulatory projection neuron 1 (MCN1) controls the gastric mill (chewing of food) central pattern generator via chemical and electrical synapses. The electrical synapse between MCN1 and the LG motor neuron contributes to the overall excitation of LG, but does not synchronize firing. We found that the electrical postsynaptic potentials (ePSPs) in LG were affected by the hyperpolarization-activated inward current (Ih): Blocking Ih increased ePSP amplitude. Computer simulations indicated that introducing Ih in either LG or MCN1 is sufficient to elicit this increase, but also resulted in a voltage-dependence such that ePSP amplitude increased with LG depolarization and decreased with LG hyperpolarization. Changes in ePSP amplitude followed the slow time course of Ih. A voltage dependence had been shown previously in the biological system (Coleman et al., Nature 378, 1995), but its mechanisms are unclear.

We thus characterized the voltage-dependence of the ePSPs, as well as its time course. The slow time course (>500 ms) plus the presence of a concomitant slow change in LG input resistance indicated that Ih may modulate current flow across the electrical synapse. Blocking Ih confirmed this: ePSP amplitudes increased at all LG membrane potentials, but more so at hyperpolarized

values, and the voltage dependence disappeared.
Our results support the idea that Ih, diminishes the electrical coupling between MCN1 and LG and causes the voltage-dependence of the MCN1 PSPs. Since Ih is modulated by several neuromodulators in this system, the voltage-dependence of this electrical synapse and thus its influence on the motor pattern may change depending on the modulatory context.

Disclosures: L.Y. Braun: None. **A.M. Yarger:** None. **W. Stein:** None.

Poster

559. **Motor Pattern Generation: Neuromodulation**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 559.28/AAA6

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG grant STE 937/8-1

Illinois State University Startup Grant

Central Illinois Neuroscience Foundation

Title: Mapping location and activity patterns of modulatory projection neurons in the stomatogastric nervous system of the crab Cancer borealis

Authors: *C. J. GOLDSMITH, W. STEIN;
Sch. of Biol. Sci., Illinois State Univ., Normal, IL

Abstract: Different sensory inputs can configure the same central pattern generator (CPG) motor circuit to produce distinct motor patterns. Little is known about the mechanisms enabling this motor pattern selection, but it is clear that control neurons in higher centers of the nervous system are involved in sensory processing and the subsequent motor pattern selection. We are addressing this issue using the well-characterized pyloric (filtering) and gastric mill (chewing of food) CPGs in the stomatogastric ganglion (STG) of the crab Cancer borealis. Several different sensory and modulatory pathways in this system elicit distinct versions of the gastric mill motor pattern that differ in the phasing of the motor neurons as well as in their firing and burst patterns. The gastric mill CPGs in the STG receives input from modulatory projection neurons (PNs) in the paired commissural ganglia (CoGs) and the oesophageal ganglion. PN activity is initiated and modulated by different sensory modalities, but only four out of about twenty CoG PNs have been sufficiently characterized to (1) identify their location, (2) show their influence on the STG motor circuits and (3) determine their activity patterns.

Each CoG has about 500 neurons and a subset of them, including the PNs, receives ascending feedback from the STG CPGs (Blitz & Nusbaum, J Neurosci 32, 2012). As a prerequisite for characterizing PN activity, we are thus mapping somata location of CoG neurons whose activity is timed with the STG motor patterns using a combination of electrophysiological recordings and voltage-sensitive dye (VSD) imaging. The VSD di-4-ANEPPS was bath-applied to the desheathed CoGs and the pyloric and gastric mill motor patterns were recorded simultaneously after stimulation of a mechanosensory pathway (Beenhakker & Nusbaum, J Neurosci 24, 2004). The cell membranes of most, if not all CoG neurons were stained and could be separated within and across focal planes, revealing distinct landmarks such as the large L-cell. More than 50 neurons in one focal plane could be imaged simultaneously. We observed pyloric-timed oscillations in the fluorescence of several neurons with a signal-to-noise ratio high enough to detect membrane potential oscillations in non-averaged recordings. The strength and phasing of the oscillations depended on the region of interest selected, indicating that individual cells can be distinguished by their fluorescence profile.
We next aim to determine if neurons in different focal planes can be imaged with a similar signal-to-noise ratio. We will then combine optical imaging data with retrograde backfill labeling to create a 3D map of CoG neuron location and activity patterns.

Disclosures: C.J. Goldsmith: None. **W. Stein:** None.**Poster**

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.01/AAA7

Topic: F.04. Neuroethology

Title: Effects of hypocretin antagonist interaction with isoflurane anesthesia on locomotor behavior in mice

Authors: *A. J. CASTANEDA[1], K. BOWYER[2], R. RYDEN[2], K. D'ANNA-HERNANDEZ[2]; [1]Psychology, [2]California State Univ. San Marcos, San Marcos, CA

Abstract: Hypocretin (HCRT), a hypothalamic neuropeptide, is involved in arousal and wakefulness (Sakurai, 2007). HCRT acts in the lateral preoptic area of the hypothalamus; an area that opposes wakefulness by regulating sleep-promoting behavior via inhibitory GABAergic connections (Szymusak & McGinity, 2008). Isoflurane anesthesia, has been shown to enhance the effects of sleep-promoting systems though GABAergic pathways (Szabdi, 2006). Though much research has been done on the effects of HCRT on wakefulness, the interaction effects of HCRT and anesthesia is relatively underrepresented. Early work suggests that an HCRT/anesthesia interaction with mice will alter the ability to regain posture on foot-paws (Kelz

et al., 2007). In this study we examine recovery from a HCRT-receptor 1 antagonist SB-334867 and the interaction effects with isoflurane anesthesia by measuring gross locomotor movement over a 30 minute time period in an open field apparatus. We hypothesized that mice given SB-334867 (30mg/kg) while under isoflurane anesthesia will exhibit significantly less locomotor movement on an open-field
test than saline injected mice while under anesthesia. Forty male mice (n=10 per group in 4 groups) were exposed to isoflurane anesthesia or no anesthesia for 3 min then given ip injections of a vehicle control or SB-334867 (30mg/kg). Animals were immediately placed in a open field apparatus for 30 minutes and locomotion monitored. The data was analyzed at separately at 10, 20 and 30 min. There was no significant effect of anesthesia, SB-334867 (30mg/kg) treatment nor
their interaction on total distance travelled during the first, second or third
10 minutes of the total 30 min test period ($p<0.05$). However, we found that mice who received only the HCRT-1 antagonist with no anesthesia spent significantly more time immobile during the first ten-minutes of testing when compared to mice which received the HCRT antagonist with anesthesia ($p>0.05$). These results suggest that there are no
long term immobility effects of the interaction between anesthesia and the downregulation of HCRT-1 activity. Anesthesia may actually be blocking the stressful effects of the injections themselves and provide beneficial effects for behavioral studies with HCRT. These results have methodological implications for further work.

Disclosures: A.J. Castaneda: None. **K. Bowyer:** None. **R. Ryden:** None. **K. D'anna-Hernandez:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.02/AAA8

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH grant R01 NS081713

Title: Effects of dorsal root stimulation on locomotor network output and V2a interneuron activity in isolated mouse spinal cord

Authors: *S. DIETZ[1], N. A. SHEVTSOVA[2], I. A. RYBAK[2], R. M. HARRIS-WARRICK[1]; [1]Neurobio & Behavior, Cornell Univ., ITHACA, NY; [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Sensory feedback is critical for modulating locomotion in response to changes in the environment or body position. Studying the effects of sensory input on the output locomotor activity and the activity of neurons potentially involved in the spinal central pattern generator (CPG) network will allow us to better understand CPG organization. The isolated mouse spinal cord permits simultaneous recordings of the output of the spinal locomotor network and the activity of identified neurons. Sensory afferents were activated by stimulation applied to the flexor-dominated (dL2) or extensor-dominated (dL5) dorsal roots during fictive locomotion evoked by bath application of NMDA and serotonin. Very low concentrations of NMDA (typically 2-3 μM), which evoked weak fictive locomotion, allowed us to obtain a variety of responses to the dorsal root stimulation. Specifically, dL2 stimulation at lower intensities typically modified the locomotor activity only within the ongoing cycle, causing an advance burst onset in the ipsilateral flexor ventral root (iL2), and burst delay in the ipsilateral extensor (iL5) and the contralateral flexor (cL2) ventral roots. The exact effect of stimulation depended on the phase of stimulus application, with maximal effects at the end of the iL2 burst before the onset of the cL2 burst. Higher-intensity dL5 stimulation evoked a multi-cycle acceleration of the motor pattern, retaining correct right-left and flexor-extensor alternation, that was often followed by a prolonged slowing or inhibition of the rhythm. To further investigate the effects of afferent stimulation we extended our previous CPG model (Zhong et al. 2012) by incorporating sensory inputs. The model was able to closely reproduce the experimentally observed effects of dL2 and dL5 stimulations and allowed us to suggest the possible organization of afferent inputs to the spinal CPG. Our earlier studies of V2a interneurons suggested that they participate in the locomotor CPG in several ways, with subsets driving ipsilateral motoneurons and subsets projecting to commissural interneurons involved in regulation of left-right alternating activity. During relatively weak dL5 stimulation applied within the 2nd and 3rd quadrants of the locomotor cycle, V2a interneurons typically received inhibitory synaptic input. During higher intensity dL5 stimulation, some V2a received prolonged inhibition. However, other V2a interneurons received excitatory drive. We propose that the V2a class encompasses subclasses with different responses to sensory input, which correspond to their different roles in the CPG. Supported by NIH grant R01 NS081713.

Disclosures: S. Dietz: None. **N.A. Shevtsova:** None. **I.A. Rybak:** None. **R.M. Harris-Warrick:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.03/AAA9

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: HSØ grant nr 22222

NFR grant nr 33333

UiO grant nr 44444

Title: Characterization of rostral medullary and pontine reticulospinal projections in the late fetal and neonatal mouse

Authors: M. S. SIVERTSEN[1], M.-C. PERREAULT[2], *J. C. GLOVER[1];
[1]Dept Physiol, Univ. Oslo, 0317 Oslo, Norway; [2]Dept of Physiol., Emory Univ., Atlanta, GA

Abstract: Reticulospinal (RS) projections represent one of the principal substrates for regulation of spinal neural networks. To provide more insight into the organization of these projections in the developing mouse, we have conducted a series of retrograde tracing studies at late fetal and early neonatal stages, focusing on reticulospinal neuron populations located in the rostral medulla and the pons. In this regard our region of interest has been rostral to the rostral limit of the raphespinal neuron population, which in developmental terms lies roughly at the boundary between rhombomeres 4 and 5.

Unilateral labeling from spinal cervical segment C1 shows that RS neurons in this region comprise a continuous population spanning the length of the rostral medulla and the pons. The population is also continuous with the main medial population of medullary RS neurons in the caudal medulla, but attains more lateral positions as it extends rostrally. The RS neurons in this region are predominantly ipsilaterally projecting, but a minority population (about 30%) projects contralaterally. Differential retrograde labeling of the two sides at C1 shows that the contralaterally projecting population lies in the more ventral, lateral, and rostral regions of the total population. The ipsilaterally and contralaterally projecting populations appear to be distinct: there is no evidence of individual RS neurons with bilateral projections. Selective labeling of defined portions of the C1 white matter shows that the majority of ispilaterally projecting RS neurons projects in the ventral funiculus and medial part of the ventrolateral funiculus. These reach the cord via the mlf as well as via trajectories lateral to the mlf. Those that project along trajectories lateral to the mlf tend to be located more rostrally. The contralaterally projecting RS neurons, by contrast, project in the lateral part of the ventrolateral funiculus.

Our findings provide a more comprehensive picture of the anatomical organization of mammalian RS projections and in particular highlight a little appreciated contralaterally projecting population of pontine RS neurons.

Disclosures: M.S. Sivertsen: None. **J.C. Glover:** None. **M. Perreault:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.04/AAA10

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: HMRI Grant 11-51

NHMRC Grant 628765

Title: Electrophysiological and molecular characterisation of long, descending propriospinal neurons in mice

Authors: *J. FLYNN[1,2], M. GOULDING[3], R. J. CALLISTER[1], B. A. GRAHAM[1];
[1]Sch. of Biomed. Sci. and Pharm., Univ. of Newcastle, Newcastle, Australia; [2]Hunter Med. Res. Inst., Newcastle, Australia; [3]Salk Inst., San Diego, CA

Abstract: Long descending propriospinal neurons (LDPNs) are spinal interneurons that project over long distances within the spinal cord. Importantly, they connect forelimb and hindlimb motor circuits, allowing for interlimb coordination. Recent evidence also shows that following spinal cord injury, intact LDPNs facilitate the formation of de novo neural pathways that bypass a lesion site and contribute to functional recovery (Bareyre et al, 2004, Nat. Neurosci. 7:269). Surprisingly, little is known about the electrophysiological and molecular properties of LDPNs. This information is needed to better understand their role in spinal cord function under both normal and pathological states.

To examine the electrophysiological properties of LDPNs, we used targeted whole-cell patch-clamp electrophysiology. Mice (C57Bl6, 22-27 days) were anaesthetized (1-3% isoflurane) and injected with a fluorescent retrograde tracer (DiI) in the right lumbar spinal cord (L2) to identify cervical LDPNs that project to this region. After 48 hours, mice were overdosed with ketamine (100 mg/kg, i.p.) and transverse spinal cord slices were obtained. Recordings (23°C, KCH3SO4-based internal) were made from fluorescently labeled LDPNs whose somata were located in upper thoracic and cervical spinal cord segments. Recordings were obtained from 12 identified LDPNs and 8 control (non-fluorescent) neurons (RMP -46.6 ± 2.7mV vs. -55.4 ± 1.4mV, Rin 602.2 ± 244.5MΩ vs. 515 ± 95.1MΩ, Mcap 27.3 ± 2.9pF vs. 15.9 ± 1.3pF). Compared to control recordings, LDPNs were more likely to discharge action potentials (APs) spontaneously (75% vs. 38%) and exhibit a tonic AP discharge pattern (80% vs. 38%) in response to depolarizing current steps (1s duration, 20 pA increments). Also, 88% of LDPNs (vs. 66% control) exhibited a depolarizing, Ih-like inward current.

To investigate the molecular phenotype of LDPNs, GlyT2-GFP and GAD67-GFP mice were labeled with Fluoro-Gold using the injection technique described above. 15% of LDPNs were GlyT2 positive (n=3 mice), while 10% were GAD67 positive (n=3 mice). Interestingly, 85% of

these inhibitory LDPNs exhibited ipsilateral or midline projections. A number of genetically defined interneuron populations (including V3, V2a, V2b, V0, and dI3) were also examined for LDPN projections. Of these, only V2b (Gata3+) neurons contributed to LDPN circuitry (8% of total LDPN population; n=1 mouse).
Our data shows LDPNs have electrophysiological properties consistent with neuronal populations known to participate in rhythmic motor behaviours. Additionally, they have inhibitory projections that are predominantly ipsilateral.

Disclosures: J. Flynn: None. **M. Goulding:** None. **R.J. Callister:** None. **B.A. Graham:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.05/AAA11

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: KAKENHI

MItsubishi Foundation

Uehara Memorial Foundation

Kao Foundation for Arts and Sciences

Title: Directional control of locomotion in the touch-evoked escape behavior in zebrafish

Authors: *K. ASAKAWA[1,2], G. ABE[1], A. MUTO[1,2], K. KAWAKAMI[1,2];
[1]Dept. of Developmental Genet., Natl. Inst. of Genet., Mishima / Shizuoka, Japan; [2]SOKENDAI, Mishima, Japan

Abstract: An animal moving through the environment determines its direction of progression based on internal and/or external sensory information. Escape behaviors of zebrafish (Danio rerio) have been good models to study directional control of locomotion because the escape direction varies flexibly and accurately depending on the position of aversive stimulus. Here, we analyzed the escape behavior of zebrafish embryos observed around 48 hour post-fertilization, which can be evoked mainly by tactile stimulation. In the touch-evoked escape, the direction is largely determined by the angle of the lateral body turn (C-bend) that occurs prior to swimming. The angle of C-bend is typically 177° and 114° against the head and tail stimulation, respectively. To identify the neural network regulating the C-bend angle, we first performed a gene trap screen to generate Gal4 lines that labeled specific population of cells in the central

nervous system. Then, by crossing with UAS-tetanus toxin light chain (TeTxLC) lines, the Gal4 lines were screened for abnormal C-bend angle phenotype. From this screen, we identified a trap line for the mafb-transcription factor gene, in which Gal4 is widely expressed in the rhombomeres 4, 5 and 6. We found that, when TeTxLC was induced in the mafb-positive domain, the embryos displayed a larger C-bend angle, thereby escaping in an abnormal direction. The increase in the C-bend angle was observed againt the head, as well as tail stimulation. In parallel, we found that the laser ablation of the Manuther cell and its homologues, the reticulospinal neurons that are activated when an escape occurs, had a minor effect on the C-bend angle. To identify the candidate neurons that is involved in the C-bend regulation, we performed the calcium imaging of the mafb-positive domain with GCaMP. We found that the mafb-positive domain contained a population of cells that were selectively activated depending on the stimulus position, as well as ones that were activated regardless of the stimulus position. The above results support the idea that the mafb-positive domain in the hindbrain can discriminate the position of the tactile stimulus and determine accordingly the C-bend angle to define an appropriate direction of locomotion.

Disclosures: K. Asakawa: None. **G. Abe:** None. **A. Muto:** None. **K. Kawakami:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.06/AAA12

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH R01

NSF GRFP

Title: Visuomotor integration in the motor cortex during skilled locomotion

Authors: *E. E. STOUT, I. N. BELOOZEROVA;
Barrow Neurol Inst., PHOENIX, AZ

Abstract: The activity of the motor cortex is essential for skilled movements, but its mechanism of response to emergent changes in the environment is poorly understood. In this study, we investigated the impact that unexpected visual and motor stimuli have on the activity of individual neurons in the motor cortex during a skilled, accuracy-dependent locomotion task. Cats were trained to walk over the evenly spaced rungs of a raised horizontal ladder. One rung was connected to a motor, and would be transiently displaced at different points along the cat's

progression. Six disturbant stimuli were applied: a motor disturbance, a visual disturbance, and an integrated visuomotor disturbance, each involving rung displacements either towards or away from the cat.

We found that the motor cortex response to these disturbances is well-defined, but that the responses of individual cortical neurons were strongly related to their anatomical and physiological characteristics. Neurons projecting to the pyramidal tract (PTNs) were more likely to respond to the disturbance than non-PTNs. PTNs with fast conduction velocities (>25 m/s) were more likely to respond than slow PTNs. PTNs receiving somatosensory information from different sources responded to different types of disturbances, at different points within the step cycle. Visual responses were observed almost exclusively in shoulder-receptive PTNs and PTNs with no receptive field.

In every neuron showing a response to the disturbances, the motor response was strongly and positively correlated with the integrated response. The visual response, when present, was always negatively correlated with the integrated response. Simple summation of motor and visual responses could not explain the integrated visuomotor response. There was little evidence of a motor cortex role in planning the adaptation to the disturbance; rather, the responses were almost exclusively concerned with on-line modification of motor commands.

We conclude that the differentiation of responses of motor cortex neurons with differing anatomical and physiological properties suggests that the functional organization of the motor cortex may be more regular and constrained than previously thought. The fact that the integrated response is not a summation of visual and motor responses raises the possibility the visual and motor streams may merge at earlier stages in motor processing. Finally, the lack of evidence for motor planning in the motor cortex suggests that that parallel processing in other motor-related structures may be occurring.

Disclosures: E.E. Stout: None. **I.N. Beloozerova:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.07/AAA13

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS080047

Title: Locomotor rhythmogenesis in the caudal hindbrain of the lamprey

Authors: *J. T. BUCHANAN[1], S. BLEL[2], M. EVANS[2], M. M. MCLOED[2];
[2]Biol. Sci., [1]Marquette Univ., Milwaukee, WI

Abstract: It is well established that the central pattern generator (CPG) for locomotion in vertebrates resides within the spinal cord. In lampreys, a lower vertebrate, the locomotor CPGs are distributed throughout the rostral-caudal extent of the spinal cord, and these neural networks generate rhythmic alternating left-right motor bursts that propagate from head to tail during forward swimming. Recently, we discovered that the motor nerves of the most caudal segment of the hindbrain, the spino-occipital (S-O) nerves, also exhibit rhythmic locomotor activity. The goal of the present study was to determine 1) whether the caudal hindbrain segment is capable of generating alternating rhythmic activity when isolated from both the rostral brainstem and the spinal cord and 2) whether the rhythmic activity of the hindbrain leads the rhythmic activity of the first spinal segment during fictive swimming.
To determine whether the most caudal segment of the hindbrain is capable of generating rhythmic activity independent of the rostral brainstem and the spinal cord, the caudal hindbrain segment hindbrain was physically isolated. The isolation was accomplished with complete transverse cuts made at the rostral border of the S-O motor column and caudally near the midpoint between the caudal S-O nerve and the first ventral root (VR). Motor activity was recorded with extracellular suction electrodes on the SO nerves. In all cases (n = 12), bath application of D-glutamate induced alternating bursting between the left and right S-O nerves in the isolated hindbrain segment. Thus, the most caudal segment of the hindbrain contains sufficient neural components for the generation of locomotor activity.
To determine whether the rhythmic activity of the caudal hindbrain leads or follows the rhythmic activity of the spinal cord, fictive swimming was induced in the isolated brainstem-spinal cord preparation by bath application of D-glutamate. Extracellular suction electrodes were used to record from the S-O nerves of the hindbrain and from the first VR and a more caudal VR of the spinal cord. Cross-correlation analysis of rhythmic bursts was used to assess the phase relationships among the nerves. During clear episodes of forward swimming in the rostral spinal cord, the S-O nerves of the caudal hindbrain burst in advance of the first VR in most preparations. Thus, the hindbrain may serve as the lead locomotor CPG in the chain of spinal locomotor CPGs.
In conclusion, the most caudal segment of the lamprey hindbrain contains a locomotor CPG which may serve as the pacemaker for the chain of spinal locomotor CPGs.

Disclosures: J.T. Buchanan: None. **S. Blel:** None. **M. Evans:** None. **M.M. McLoed:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.08/AAA14

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: International Foundation for Research in Paraplegia

Craig H Neilsen

Title: Brainstem control of trunk and hindlimb motoneurons during fictive locomotion in the neonatal mouse

Authors: C. JEAN-XAVIER, *M.-C. PERREAULT;
Emory Univ. Sch. Med., Atlanta, GA

Abstract: Efficient and fluent locomotion in limbed animals requires appropriate coordination of trunk and hindlimb muscles. We have shown that the brainstem reticular formation, via the medullary reticulospinal system, is organized to provide both independent and integrated control of trunk and hindlimb motoneurons (MNs) (Szokol et al 2008, Szokol and Perreault 2009). How the reticulospinal system controls the two sets of MNs during ongoing motor activity is however not well understood. Here we use an ex vivo brainstem-spinal cord preparation of the neonatal mouse (postnatal day (P) 0 - P2) to examine the pattern of activity of trunk and hindlimb MNs during fictive locomotion, both before and after removal of supraspinal inputs.
Fictive locomotor activity was induced by pharmacological activation of the spinal locomotor networks (dopamine 50 µM; serotonin 10 µM; N-Methyl-D-aspartate 5 µM), and monitored using calcium imaging to ascertain that the MNs recorded belonged to the medial motor column (MMC) controlling trunk muscles or the lateral motor column (LMC) controlling hindlimb muscles. In the brainstem-attached preparation, rhythmic locomotor activity in MMC MNs overlapped with rhythmic locomotor activity in ipsilateral LMC MNs but alternated with the rhythmic locomotor activity in contralateral MMC MNs. Typically, MMC MNs displayed a double-burst during each locomotor cycle which was about 20% longer than the burst in LMC MNs (4.3 ± 0.1 s vs 3.4 ± 0.4 s, n=4). Removal of the brainstem decreased the locomotor cycle duration by about 18 % from 6.5 ± 0.5 s to 5.3 ± 0.6 s, and the burst duration in MMC and LMC MNs by about 10 % and 20%, respectively.
Our results are in agreement with the idea that descending control of locomotion is operational already by birth (Gordon and Whelan 2008) and further suggest that modulation of trunk activity is an important part of this control.

Disclosures: C. Jean-Xavier: None. **M. Perreault:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.09/AAA15

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Karolinska Institute

Swedish Research Council

European Union (FP7)

Title: Optogenetic activation of V2a interneurons produces locomotion in zebrafish

Authors: *J. AUSBORN, E. EKLÖF LJUNGGREN, S. HAUPT, A. EL MANIRA; Karolinska Inst., Stockholm, Sweden

Abstract: Neural networks in the spinal cord can generate locomotion in the absence of rhythmic input from higher brain structures or sensory feedback. The ability of the spinal networks to produce rhythmic locomotor patterns originates from the capacity of the excitatory interneurons to provide the necessary drive to other spinal network neurons. The identity of these spinal interneurons underlying the excitatory drive within the locomotor circuit has, however, remained unclear. We have previously shown that the V2a interneurons represent an intrinsic source of excitation necessary for the generation of swimming movements in larval zebrafish. In the present study, we utilize optogenetic tools to determine if activating the V2a interneurons is sufficient to produce swimming activity. We used a transgenic zebrafish line that selectively expresses Gal4 to drive the expression of channelrhodopsin-2 (ChR2) in V2a interneurons. To make sure that Gal4 expression is limited to V2a interneurons, we used a specific antibody for Chx10, a transcription factor expressed in these interneurons. We then performed whole-cell patch-clamp recordings from ChR2-expressing V2a interneurons. Blue light stimulation induced a large depolarization associated with a burst of action potentials. In spinalized larval zebrafish, blue light stimulation was sufficient to produce coordinated swimming activity. This optogenetically induced motor pattern displays left-right alternation and a rostro-caudal delay, which are characteristic of swimming in intact zebrafish. Blocking glycinergic transmission transformed the left-right coordinated activity into synchronous bursting on both sides. Our results indicate that the V2a interneurons provide the excitatory drive to initiate and maintain the swimming activity. These interneurons can thus be considered a sufficient intrinsic source of excitation within the spinal locomotor circuits.

Disclosures: J. Ausborn: None. **E. Eklöf Ljunggren:** None. **S. Haupt:** None. **A. El Manira:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.10/AAA16

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Swedish Research Council

European Union (FP7)

Karolinska Institute

Title: Functional connectomics of fast and slow locomotor microcircuits in adult zebrafish

Authors: *K. AMPATZIS, J. SONG, J. AUSBORN, A. EL MANIRA;
Karolinska Institutet, Dept. of Neurosci., Stockholm, Sweden

Abstract: Locomotor movements are produced by an intricate processing within the spinal networks that results in the sequential activation of motoneurons. It is, however, still unclear if these networks are composed of uniformly connected circuits, or if they are subdivided into multiple microcircuits. In adult zebrafish, we previously showed that only secondary motoneurons (sMNs) participate during swimming, while primary MNs (pMNs) are active only during escape. At swimming frequencies up to 12-15 Hz, only slow and intermediate sMNs are recruited, while fast sMNs become recruited at higher frequencies. We also showed that V2a interneurons represent a major source of excitation within the spinal locomotor network, and display a frequency-dependent recruitment pattern similar to that observed in MNs. In this study, we set out to determine if the different V2a interneurons are uniformly connected to all MNs or if they are preferentially connected to MNs recruited within the same frequency window. For this we deploy dual whole-cell patch-clamp recordings from identified slow, intermediate and fast MNs and V2a interneurons. Our results show that V2a interneurons that are recruited during fast swimming make strong monosynaptic connections with fast sMNs and pMNs. These connections comprise both chemical glutamatergic transmission and electrical gap-junctions. In contrast, fast V2a interneurons make very weak or no synaptic connections with slow and intermediate sMNs, which instead receive strong chemical monosynaptic connections from V2a interneurons that are recruited during slow/intermediate swimming frequencies. During swimming, the peak activity of V2a interneurons and sMNs recruited at slow/intermediate swimming is always occurring before that of V2a interneurons and MNs that are recruited at faster frequencies. In addition, the activity of the connected V2a interneurons and MNs is tightly coupled with firing of V2a interneurons, always preceding that of corresponding MNs. These results suggest that the locomotor network is composed of multiple microcircuits consisting of preferentially connected V2a interneurons and MNs that are recruited within the same frequency window during swimming.

Disclosures: K. Ampatzis: None. **J. Song:** None. **J. Ausborn:** None. **A. El Manira:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.11/AAA17

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NINDS Grant R01-NS067299

The Searle Foundation

The Esther A. and Joseph Klingenstein Fund

The Alfred P. Sloan Foundation

Title: Speed dependent shifts in the ratio of excitation to inhibition shape motoneuron recruitment in larval zebrafish

Authors: *S. KISHORE[1], M. W. BAGNALL[2], D. L. MCLEAN[2];
[1]NBP, [2]Neurobio., Northwestern Univ., Evanston, IL

Abstract: Movements of increasing intensity are produced by the progressive recruitment of larger motor units. Motoneuron size and input resistance (Rin) are thought to play a significant role in determining recruitment order, but the relative contribution of these intrinsic cellular properties, versus differences in premotor synaptic input, in governing recruitment during locomotion is harder to assess. Using cell attached and whole cell voltage-clamp recordings from motoneurons in the spinal cord of larval zebrafish, we tested two possible explanations for the observed order of recruitment: (1) That the entire motor pool receives similar levels of excitation and inhibition, making Rin the primary determinant in recruitment order; or (2) that levels of excitation and inhibition vary across the motor pool in a systematic fashion, such that differences in premotor synaptic input primarily determine recruitment order. We find that at slow swim speeds, all motoneurons receive approximately equal amounts of peak excitatory current. Consequently, high Rin motoneurons are recruited, while low Rin motoneurons remain below threshold, consistent with the hypothesis that cellular excitability governs recruitment at slow speeds. In contrast, as swim speed increases, excitatory synaptic current increases far more for low Rin motoneurons than for high Rin motoneurons (i.e., the excitatory synaptic gain is inversely proportional to Rin). As a result, the bigger increases in synaptic input selectively engage low Rin motoneurons at higher swim speeds, consistent with the hypothesis that

differences in premotor synaptic input can also govern recruitment. We next examined whether synaptic inhibition follows a similar pattern as excitation. At slow swim speeds inhibitory synaptic currents are similar across all neurons, consistent with the postsynaptic intrinsic hypothesis. However, in contrast to excitation, inhibition increases with increasing swim speed across all motoneurons, indicating that it may not be as selective as excitation. The net result of these synaptic data is that for high Rin motoneurons, the excitation/inhibition (E/I) ratio actually decreases with increasing swim speed, eventually turning off firing at the highest speeds; but in low Rin motoneurons, the E/I ratio increases with increasing swim speed, allowing those cells to be recruited effectively. Our data suggest that spinal rhythm generating circuitry transitions from an intrinsic strategy based on postsynaptic excitability at slow speeds, to a network-based strategy based on differential premotor synaptic input at higher speeds.

Disclosures: S. Kishore: None. **D.L. McLean:** None. **M.W. Bagnall:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.12/AAA18

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS067299

Title: Motoneuron excitability shapes vision-based action selection in the spinal cord

Authors: *W.-C. WANG, D. L. MCLEAN;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: The recruitment of motoneurons in the spinal cord follows Henneman's size principle. When generating increasing force, smaller motoneurons with higher input resistances are recruited first, while larger motoneurons with lower input resistances are added to the active pool later. However, little is known about how descending inputs from the brain utilize this organization to select different movements in response to sensory stimuli. In 4-6 day old larval zebrafish, spinal motoneurons are arranged dorsal-ventrally according to their size, input resistance, and recruitment order during swimming. We find that a sudden increase in light elicits low frequency swimming and more reliably activates smaller, ventral motoneurons with higher input resistances. Based on this observation, we next asked how this light-elicited, differential recruitment pattern of spinal motoneurons is achieved. The nucleus of the medial longitudinal fascicle (nMLF) is a compact cluster of descending neurons in the midbrain, which is activated by visual stimuli and is thus a good candidate for relaying visual information to the spinal cord.

Unlike spinal motoneurons, neurons in the nMLF respond to the light stimulus more homogeneously with similar reliability. By examining the anatomy and physiology of identifiable nMLF neurons, we demonstrate that one of the nMLF neurons (the caudal medial-lateral neuron, MeLc) synapses onto both larger and smaller spinal motoneurons. MeLc-elicited EPSPs in motoneurons with input resistances ranging from 30-600MΩ have similar amplitudes, but different waveforms. The slower decay of the EPSP waveform in higher input resistance motoneurons permits temporal summation of EPSPs, which allows small motoneurons to reach threshold more easily. Thus, the size principle helps explain the preferential activation of motoneurons and the selection of slow swimming movements in response to visual stimuli.

Disclosures: W. Wang: None. **D.L. McLean:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.13/AAA19

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant DC012536

NIH Grant NS067299

Title: Spinal axial microcircuits provide evolutionary template for limb control

Authors: *M. W. BAGNALL[1], Y. KIMURA[2], S.-I. HIGASHIJIMA[2], D. L. MCLEAN[1]; [1]Neurobio. & Physiol., Northwestern Univ., Evanston, IL; [2]Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Neural circuits controlling vertebrate limbs are thought to have evolved from fish axial circuitry, but the nature of this transformation is unknown. How did the axial circuit reorganize to produce flexor and extensor motor and premotor circuits? Using physiological recordings from motor neuron pairs in vivo, we show that in fact the zebrafish axial circuit is composed of two largely distinct parallel microcircuits, producing independent control of dorsal and ventral musculature. Both excitatory (V2a/Chx10+) and inhibitory (V1/En1+) premotor circuits are segregated into these two streams. We further demonstrate that fish rely on differential control of dorsal and ventral musculature for vital postural movements. We conclude that parallel microcircuits governing dorsal and ventral musculature, which originally developed to facilitate postural behaviors, are likely templates for the evolution of extensor/flexor control in

limbed vertebrates. Reweighting of existing connectivity, rather than extensive reorganization, is thus a strong candidate mechanism for this evolutionary transformation.

Disclosures: M.W. Bagnall: None. **Y. Kimura:** None. **S. Higashijima:** None. **D.L. McLean:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.14/AAA20

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH DP OD006411

Title: Dendritic filopodial dynamics of motoneurons in larval zebrafish during the day and night

Authors: *J. V. DIPIETRO, JR[1], J. R. FETCHO[2];
[1]Neurobio. and Behavior, [2]Cornell Univ., Ithaca, NY

Abstract: The dendrites of neurons are highly dynamic structures. Throughout development, dendrites extend and retract filopodia as they search for new synaptic inputs. Some filopodia are stabilized by synapse formation, while previously stabilized filopodia may retract resulting in removal of synapses and dendritic pruning. Understanding dendritic filopodia dynamics may provide clues about how the nervous system homeostatically regulates the formation and breaking of synaptic contacts throughout the circadian cycle, during sleep and wake, and across development. The genetic and optical techniques available for zebrafish also allow for analysis of these dynamics in individual neurons. We used two-photon microscopy to take high-resolution three-dimensional time series images of single zebrafish primary motoneurons in vivo, giving us the opportunity to analyze all of the processes of individual neurons in an identifiable class. Because the animal remains intact during the imaging, the same cell can be analyzed at multiple times. The entire dendritic arbor of individual cells (one cell per fish, n=9) was imaged over the course of 15 minutes (6 z-stacks, 3 minutes apart), once during the day and once at night. Fish were light shifted at birth and age matched to account for order-of-imaging and age related effects. We find that the level of dendritic dynamics remains similar in day and night, without a precipitous drop or rise in overall dynamics during the day or night in association with changes in motor activity. Our early evidence, however, supports the conclusion that the balance of extensions and retractions may shift during day and night, with the ratio of retractions to extensions being greater at night than during the day. This has potential implications for how

patterns of formation and removal of synaptic connections are shaped in relation to changes in overall levels of motor activity or behavioral state during day and night.

Disclosures: J.V. Dipietro: None. **J.R. Fetcho:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.15/AAA21

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant R01-NS26539

NIH Grant F32-NS083099

Title: Functional organization of post-migratory facial motor neurons in zebrafish

Authors: *K. L. MCARTHUR, J. R. FETCHO;
Neurobio. & Behavior, Cornell Univ., Ithaca, NY

Abstract: The time and place of a neuron's birth set the stage for its functional development, but many neurons migrate away from their places of origin before and during periods of initial circuit formation. In order to understand the interplay between neuronal migration and development, we are exploring the functional properties of facial branchiomotor neurons (FBMNs) in zebrafish hindbrain, which undergo a dramatic caudal migration from rhombomere 4 to rhombomeres 6 and 7 in wild type embryos. In a previous study, we used whole-cell patch clamp recordings and dendritic dye fills to demonstrate the existence of physiological and morphological heterogeneity in the FBMN post-migratory population. We present here additional data pertaining to the functional organization of the FBMN population. Using a newly generated transgenic line (zCREST1:Dendra) in which FBMNs express a photo-convertible fluorescent protein, we show that post-migratory FBMNs establish a clear age-related organization, wherein dorsal cells are younger than ventral cells (similar to the age order of hindbrain interneurons). Using a second transgenic line (zCREST1:GCaMP5) in which FBMNs express a genetically encoded calcium indicator, we confirm and extend our previous observation of functional heterogeneity across FBMNs, with only a subset of neurons exhibiting smaller spontaneous bursts (0.5-1Hz) in addition to larger tail movement-related bursts. Further, we observe that neither bursting pattern appears until migration has been terminated, suggesting that FBMNs do not receive functional synaptic input until they reach their final destination. The next step in this project will be to compare this wild type functional topography to that of mutant

zebrafish in which FBMN caudal migration is specifically disrupted, to determine if the functional organization of this population is maintained despite the aberrant location of its cell bodies.

Disclosures: K.L. McArthur: None. **J.R. Fetcho:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.16/AAA22

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant DP OD006411

Title: *In vivo* kinetics of glycine receptor re-distribution

Authors: *D. M. CHOW, J. R. FETCHO;
Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: The regulation of neurotransmitter receptor insertion and removal from synaptic sites plays an important role in synaptic plasticity. Despite the longevity of individual synapses, bound synaptic proteins exchange constantly with a cytosolic pool. Furthermore, the dynamic equilibrium maintained between insertion and removal of synaptic components influences receptor concentration and synaptic weighting. Understanding the regulation of receptor turnover may yield insights into how neurons regulate changes in synaptic plasticity and tune the relative weights of their inputs. Receptor turnover, however, is little studied in vivo due to the difficulty of visualizing synaptic proteins in intact preparations. We chose to study the re-distribution dynamics of the glycine receptor, the pre-dominant inhibitory neurotransmitter receptor in the spinal cord, in primary motoneurons of the larval zebrafish. The transparency of the larval zebrafish has allowed us to monitor the distribution of glycine receptors in single neurons of an intact living animal. We stochastically expressed glycine receptor α1 subunit tagged with the photo-convertible fluorescent protein dendra in isolated motoneurons. By precise targeting with converting pulses from a UV laser followed by two photon imaging, we have been able to quantify the rates of receptor turnover at single synaptic puncta. Our analysis indicates that the exponential time constant for turnover in primary motoneurons is on the order of 10 hours. We hope that by determining the kinetics of receptor turnover at defined synaptic contacts in vivo we may be able to link regulation of receptor turnover with synaptic properties under physiological conditions.

Disclosures: D.M. Chow: None. **J.R. Fetcho:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.17/AAA23

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: HFSP LT001234/2011-L

NSF IIS-0904594

Title: Optogenetic rostro-caudal inhibition of movement without inhibition of afference in the spinal cord of mice

Authors: *V. CAGGIANO, M. SUR, E. BIZZI;
MIT, Cambridge, MA

Abstract: The γ-aminobutyric acid (GABA) and glycine are the main inhibitory neurotransmitters in the adult mammalian spinal cord. Several line of research have supported the idea that inhibitory interneurons have a local organization defined by sensory terminals which contribute to control motor neurons for limb alternation/coordination and for regulation of sensory-motor reflexes.
In order to understand the role and the organization of inhibitory neurons in the control of motor and sensory information within the spinal cord with both higher precision in space and in time, we developed a new method to optogenetically manipulate the spinal circuits in anesthetized and in awake freely moving animals using transgenic mice expressing ChR2 (Channelrhodopsine-2) in GABAergic/glycinergic neuronal populations (VGAT-mhChR2).
In anesthetized animals, light was shined at different levels of the spinal cord (from middle thoracic to middle lumbar with about 0.5 - 2 mm steps) by means of a movable optical fiber. Movements produced by electrical stimulation of the motor cortex were strongly reduced when light stimulation was applied (about 90% reduction, $p < 0.05$ sign-rank test, $n = 10$ mice). Maximum suppression was obtained at the middle-lower thoracic sector. These observation were confirmed by testing motor behavior in freely moving animals when light was shined above T12. Brief periods of optical stimulation of the spinal cord produced a loss of muscular tone (median latency of EMG suppression 6.55 ms) in all of the muscles i.e. agonist and antagonist, caudal to the stimulated point.
Some of the animals from the previous experiment were tested to evaluate the sensory consequences of the inhibition of the motor behavior. Once we identified the muscle(s)

suppressed during light stimulation, we anesthetized the animal and we recorded single neuron activity in the somatosensory cortex evoked by electrical stimulation of one of the muscles affected. In addition, stimulation of the hind-paw/foot was performed. Overall, the cortical sensory responses evoked by peripheral electrical stimulation showed the same pattern (time and amplitude) with only electrical and electrical-couple-optical stimulation of the spinal cord.
In conclusion, we developed a new technique for investigating the spinal inhibitory network in both anesthetized and awake freely moving mice. These experiments showed a new global rostrocaudal inhibitory effect without modifying the sensory information transmitted to the cortex at the middle/lower thoracic level in addition to the well-established local inhibition for postural control and locomotion which is present at the lumbar and sacral levels.

Disclosures: V. Caggiano: None. **M. Sur:** None. **E. Bizzi:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.18/AAA24

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Localization of glycinergic neurons selectively activated during paradoxical (REM) sleep in the rat: their potential role in muscle atonia

Authors: *S. VALENCIA GARCIA, O. CLÉMENT, P. A. LIBOUREL, S. ARTHAUD, P. H. LUPPI, P. FORT;
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Abstract: During paradoxical sleep (PS), somatic motoneurons are strongly inhibited causing a loss of tone in the whole skeletal musculature, a distinctive motor event of this sleep state. As revealed by intracellular recordings in the cat, glycine is the main inhibitory neurotransmitter responsible for the sustained hyperpolarization of both masseter and lumbar motoneurons during PS. Our current functional model thus assumes that glutamatergic PS-on neurons of the pontine sublaterodorsal nucleus (SLD) generate PS atonia by means of excitatory efferent projections to glycinergic premotoneurons. However, the exact location within the brainstem and/or spinal cord of these PS-on glycinergic neurons still remains a matter of debate. To bridge this gap, we used three experimental groups of rats prepared for polysomnographic recordings (EEG and EMG): 1) control (PSC); 2) deprived for PS during 72h with the flower pot technique (PSD, n=4); and 3) allowed to recover for 150 minutes of such deprivation during which they experienced ≈40% of PS (PSR, n=4). Ten days before PS deprivation, PSR rats were submitted to a retrograde tracer

Fluorogold (FG) injection into the ventral spinal cord at T13-L1 levels. An additional control group included rats forced to walk for 2h (STEP, n=4) in order to activate neurons involved in locomotion. Brainstem and spinal cord sections were then processed for double labeling combining immunohistochemistry of Fos (used as a marker of neuronal activity) with non-radioactive in situ hybridization of GlyT2 mRNA (the specific neuronal glycine reuptake transporter). Other sections from PSR rats were submitted to Fos/FG double immunostaining. Double-labeled Fos+/GlyT2+ neurons were quite exclusively observed in the ventral medulla oblongata of PSR rats. They were localized in the lateral paragigantocellular, alpha (Giα) and ventral gigantocellular (GiV) and raphe magnus nuclei (RMg). In the GiV, almost 80% of Fos+ neurons expressed GlyT2, whereas there are respectively 70% and 60% Fos+/GlyT2+ neurons in the Giα and RMg. At lumbar level, occasional Fos+/GlyT2+ neurons were seen in PSC, PSD and PSR rats while they were numerous in STEP rats with sustained locomotor activity. Finally, Fos/FG double immunostaining in PSR rats unraveled that a large number of Fos+ projecting to the ventral spinal cord is present only in the GiV.
These results highly suggest that GiV glycinergic neurons selectively activated during PS project directly to the spinal cord and are responsible for the hyperpolarization of somatic spinal motoneurons during this state.

Disclosures: S. Valencia Garcia: None. **O. Clément:** None. **P.A. Libourel:** None. **S. Arthaud:** None. **P.H. Luppi:** None. **P. Fort:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.19/AAA25

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: WCU program of the Korean NRF (grant number R32-2008-000-10218-0)

Basic Science Research Program of the Korean NRF (2012R1A1A2006996)

Title: Bursts of spikes driven by T-type calcium channels occur in motor but not visual thalamocortical neurons in brain slices

Authors: H. R. KIM, S. Z. HONG, *C. D. FIORILLO;
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Abstract: Within an intact nervous system, neurons can be classified as either sensory or motor based on anatomy and physiological responses. However, it is not known whether there is any

systematic difference between the neurons of sensory versus motor systems at the cellular or molecular level. Based on theoretical considerations, we formulated the hypothesis that motor but not sensory neurons generate “all-or-none” bursts of action potentials under natural conditions. A major challenge in testing this hypothesis is that different types of neurons display differences in the electrical properties of their membranes that are unrelated to any distinction between sensory and motor. Although it would be desirable to systematically compare a large diversity of sensory and motor neurons in order to overcome this variability, we have taken the modest initial step of comparing sensory versus motor thalamus. We chose thalamus because it has both sensory and motor divisions, and yet thalamocortical neurons are known to share many common features, including the expression of T-type calcium channels that are capable of generating bursts of spikes under certain conditions.
Whole-cell current clamp recordings were made in the dorsal lateral geniculate nucleus (LGNd) (visual) and ventral lateral nucleus (VL) (motor) in brain slices from 3-4 week old rats. Depolarizing currents of 10 ms duration were increased in small increments from potentials of -70 and -80 mV. At -70 mV, when most T-type channels were inactivated, no difference was observed between LGNd and VL in the number of evoked sodium spikes. From -80 mV, we observed a “low threshold spike” (LTS) mediated by T-type calcium channels. When the LTS was evoked by a minimal depolarizing current, it often caused just 0 or 1 spike in LGNd (0.9±0.2, mean±sem, n=31), but 2 or more in VL (2.5±0.3, n=48), indicating that it had an all-or-none burst property in VL but not LGNd. The maximal number of spikes (evoked by large 10 ms current injections) was also greater in VL than LGNd. A second experimental protocol used dynamic clamp to evoke the LTS under more natural conditions by injecting artificial excitatory postsynaptic conductances (EPSCs) at various times after hyperpolarization to -80 mV by injection of a chloride-like conductance. Once again more spikes were found in VL. We often observed 2 or more spikes in response to a single EPSC in VL (7 of 14 neurons), but never in LGNd (0 of 26 neurons). In conclusion, our comparison of two thalamic nuclei supports the hypothesis that motor but not sensory neurons generate bursts of spikes.

Disclosures: H.R. Kim: None. **S.Z. Hong:** None. **C.D. Fiorillo:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.20/AAA26

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH 5R01-HL104127-04

Title: Laser ablation of Dbx1 pre-Botzinger interneurons impairs and then precludes respiratory rhythm generation

Authors: *C. A. DEL NEGRO[1], X. WANG[1], M. C. D. PICARDO[1], J. A. HAYES[2];
[1]Dept. of Applied Sci., The Col. of William & Mary, Williamsburg, VA; [2]Neurobiologie et Developpement, Inst. de Neurobiologie Alfred Fessard, Gif-sur-Yvette, France

Abstract: Neurons whose progenitors express Dbx1 may comprise the excitatory core of the preBotzinger complex (preBotC), a rhythmogenic site essential for breathing behavior in mammals. We dub this the Dbx1 core hypothesis, and it predicts that cumulative destruction of Dbx1+ neurons in the preBotC should degrade its rhythmicity and ultimately lead to irrevocable rhythm cessation. We used a cell-specific laser detection and ablation methodology to selectively destroy Dbx1+ neurons in slice preparations that retain the preBotC as well as hypoglossal (XII) motoneurons and premotor neurons, and thus encapsulate a complete respiratory motor circuit and in vitro model of the behavior. Ablating Dbx1+ neurons in sequence progressively diminished the frequency, amplitude, and regularity of XII output. The rhythm stopped irreversibly after 85±45 ablations, which corresponds to ~26% of the minimum estimated population size. Neuropeptide stimulation evoked transient but unsustainable XII output in lesioned slices. These data support the hypothesis that Dbx1+ neurons comprise the preBotC rhythmogenic core, and further show that Dbx1+ neurons may play a premotor role. Dismantling the Dbx1+ core piecewise precludes rhythm-generating functionality in the preBotC without completely destroying the underlying network. Our results clarify the genetic cellular core of a mammalian central pattern-generating circuit and quantify the parameters that govern its functionality.

Disclosures: C.A. Del Negro: None. **X. Wang:** None. **M.C.D. Picardo:** None. **J.A. Hayes:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.21/BBB1

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF-IOS 1022442

Title: Ontogeny and plasticity of GABAergic inhibition in respiratory control

Authors: *E. A. MACMURRAY, C. M. CARTAGENA DE JESUS, B. E. TAYLOR;
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Abstract: The tadpole brainstem preparation is a facile model for studying the isolated, yet intact, central network that controls vertebrate breathing. γ-aminobutyric acid (GABA) signaling plays a role in this neural network, as it does in many rhythmically oscillating networks. Previous studies depict an interaction between ethanol and GABA receptors; however, few have investigated this interaction with respect to respiratory control. Sudden Infant Death Syndrome (SIDS), which is characterized by respiratory failure, is associated with deficits in serotonergic and GABAergic systems, and its incidence increases with prenatal and postnatal alcohol exposure. We are interested in developmental changes in GABAergic inhibition and critical developmental periods in which ethanol can modulate GABAergic activity. We hypothesized that respiratory GABAergic inhibition varies with development and that early chronic ethanol exposure attenuates the GABA response during specific developmental periods. Preliminary analyses of cranial nerve output before and after bath application of 0.25 mM GABA suggest that this traditionally inhibitory signaling increases the frequency of lung-breathing in early tadpole development. Ethanol modifies the effect of GABA in pre-metamorphic but not post-metamorphic tadpoles. Additionally, preliminary results suggest that nonchloride-mediated GABA signaling is the primary mode of GABAergic inhibition underlying early tadpole lung breathing. By further understanding the developmental changes in GABAergic inhibition, we hope to delineate critical developmental periods that may be useful targets in the study of respiratory disease.

Disclosures: E.A. Macmurray: None. **C.M. Cartagena De Jesus:** None. **B.E. Taylor:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.22/BBB2

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS72211

NIH Grant HL70029

Title: The RTN and pFRG are functionally separate nuclei in the control of respiration

Authors: *R. T. HUCKSTEPP, K. P. CARDOZA, L. E. HENDERSON, J. L. FELDMAN;
Neurobio., UCLA, Los Angeles, CA

Abstract: A distinct respiratory oscillator driving active expiration appears to be located close to the facial nucleus in the regions of the retrotrapezoid nucleus (RTN) and the parafacial respiratory group (pFRG). Here, we address whether these two nuclei have distinct functional roles in central pattern generation for breathing in adult rat. Neurons in each region were transfected with adeno-associated viruses (AAVs) containing inserts for either the allatostatin or HM4D (DREADD) receptor. The RTN and pFRG were silenced independently, with allatostatin or CNO respectively, under conditions where expiration was active, i.e., during hypoxia, hypercapnia, and disinhibition (with bicuculline and strychnine) of the pFRG. We found that the pFRG and RTN had different roles in the patterning of breathing. Silencing the RTN altered tidal volume and the amplitude of EMGs of inspiratory- and expiratory-related muscles. Disinhibiting the pFRG altered respiratory frequency by increasing expiratory duration. Silencing the pFRG reduced expiratory-related abdominal muscle EMG without affect on inspiratory-related muscle EMGs. We suggest that the RTN affects the overall drive to breathe, with the pFRG a key element of the expiratory oscillator.

Disclosures: R.T. Huckstepp: None. **K.P. Cardoza:** None. **L.E. Henderson:** None. **J.L. Feldman:** None.

Poster

560. **Motor Pattern Generation: Vertebrate Models I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 560.23/BBB3

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Beta Beta Beta National Biological Honor Society

Research-Enriched Academic Challenge, SIUC

Southern Illinois University School of Medicine

Title: Functional loss of peripheral nerve conduction following embryonic pyridoxine administration in chick

Authors: *Y. FEDOROVICH[1], A. A. SHARP[2];
[1]Physiol., [2]Anat., Southern Illinois Univ. Sch. of Med. Carbondale, Carbondale, IL

Abstract: Our long term goal is to understand how sensory information is used during embryogenesis to modulate motor behavior and establish normal sensorimotor circuitry. Towards this end, we have been studying the effect of embryonic administration of pyridoxine

(vitamin B6) on chick embryos. Studies in adult mammals have shown that large doses of pyridoxine cause large fiber sensory neuropathies accompanied by ataxia. While pyridoxine mostly kills muscle proprioceptive neurons, there is still a significant loss of some skin innervating neurons in mammals. Previously, our lab has shown that embryonic administration of pyridoxine in chick on embryonic day (E) 7 and E8 causes an alteration of leg movement on E9 as well as post hatch. Anatomical examination of this lesion on E13 reveals a significant decrease in the number of large diameter axons in the N. Tibialis, a 70-100% decrease in trkC positive dorsal root ganglion (DRG) neurons, no effect on substance-P and CGRP positive DRG neurons, and no effect on the number of motor neurons. These data suggest that pyridoxine toxicity is largely restricted to proprioceptive neurons innervating muscle in chick. The goal of the current study is to examine the functional effects of embryonic pyridoxine lesion. Specifically, we hypothesized there would be a decrease in the amplitude of the rapid component of evoked compound action potentials (CAPs) in N. Ischiadicus (analogous to the mammalian sciatic nerve) corresponding to a loss of large diameter proprioceptive axons. Animals were incubated in a forced-air incubator. The treated and control animals were injected with either 3.75 mg of pyridoxine in 100 µl of physiological saline or 100 µl physiological saline, respectively, through an opening made in the shell on E7 and E8. At E13, embryos were removed and placed into oxygenated physiological saline. The N. Ischiadicus was exposed at the point of its formation by the Ischiadic plexus and stimulated with current pulse via a suction electrode. The resulting CAPs were recorded distally before the nerve bifurcated proximal to the popliteal fossa with another suction electrode. We have observed that pyridoxine causes a decrease in the amplitude of the first peak and trough of the CAP, but only the decrease in trough amplitude is significant. This finding is consistent with our anatomical studies indicating that pyridoxine kills proprioceptive neurons innervating muscle. We are currently examining the effects of pyridoxine on CAPs in skin and muscle innervating nerves to assess the specificity of the lesion and CAPs in younger embryos to delineate the onset of functional loss.

Disclosures: Y. Fedorovich: None. **A.A. Sharp:** None.**Poster**

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.01/BBB4

Topic: D.14. Cerebellum

Support: DFG-SU171

Title: Similarities and differences in the neuronal wiring of the cerebellar nuclei of rodents and primates

Authors: S. HAMODEH, *F. R. SULTAN;
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Abstract: The deep cerebellar nuclei (DCN) undergo marked changes during the evolution of mammals and especially in primates. It is little known whether and how these morphological changes relate to changes in the cellular architecture of the DCN and also how these changes may lead to an added vulnerability in humans leading to neurodegenerative diseases such as Friedreich´s ataxia. Addressing this question has been hindered by the limited scope of methods so far to compare the neuropil in phylogenetically diverse species. In this study we quantified the components of the neuropil of the rhesus monkey's deep cerebellar nuclei (DCN) to compare it to the results we have previously obtained in rats. We obtained 3D immunostained samples that were segmented, reconstructed and quantified with a 3D quantitative immunohistochemical method. The method was sufficiently fast enough to cover the different subregions of the DCN. The neuropil was either stained with an antibody against dendrites (microtubule associated proteins, MAP2a, b) or against the Purkinje cell axons (PCP2 antibody). We obtained 512 probes sampling from a total volume of 163.8mm3. We observed systematic differences in fiber length density, average fiber diameters and volume fraction within different parts of the DCN. In the rat the dendritic and axonal (PCP2) fiber diameters were highest in the phylogenetically older medial and anterior interposed nuclei (MN and AIN), whereas the fiber density was higher in the newer posterior interposed and lateral nuclei (PIN and NL). In the rhesus monkey a similar picture emerged with the exception that the dendritic diameters within the PIN were as large as those in the AIN and NM. This could indicate that the PIN neurons are scaled up in the larger rhesus monkey cerebellum. In contrast, this effect was not seen in the NL. This observation could point towards a cellular mechanism that leads to the gyral folding observed within the primate NL. A detailed mapping on the NL surface will allow us to further test this hypothesis.

Disclosures: S. Hamodeh: None. **F.R. Sultan:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.02/BBB5

Topic: D.14. Cerebellum

Support: BBSRC studentship BB\F017146\1

Title: Form and function in the cerebellar noradrenergic and serotonergic systems

Authors: M. LONGLEY, *C. H. YEO;
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Abstract: Noradrenaline (NA) and 5-HT are important for learning and memory across several brain regions. The cerebellum is crucial for certain forms of motor learning including classical conditioning of the eyeblink response and cerebellar β-adrenoceptor function is essential for its consolidation (Paredes et al, Neur Learn Mem 92:267,2009), consistent with an earlier theory that NA may provide the consolidation signal (Gilbert, Nature 254: 688,1975). As the next step in understanding their roles, we here analyze the detailed anatomy of these monoaminergic afferent systems and the distribution of β-adrenoceptors in the cerebellum.
Immunofluorescent labelling of the noradrenaline reuptake transporter (NET) and serotonin reuptake transporter (SERT) revealed strong, orthogonal anisotropies in the NA and 5-HT afferent fibres throughout the cortical molecular layer in rats. In the vermis, NA fibre trajectories were sharply confined to the parasagittal plane with very restricted transverse extensions, suggesting they travel within the parasagittal zones and, possibly, the microzones of the cerebellar cortex. In sharp contrast, 5-HT fibres of the vermis travelled extensively in the transverse plane with very restricted trajectories rostro-caudally. This pattern of intrazonal NA and transzonal 5-HT afferent trajectories was seen in all regions of the cortex, including the hemispheres.
Both NA and 5-HT are released from multiple varicosities along every fibre. Their distributions indicate that individual NA fibres may be capable of modulating activity within a zone or even within a microzone but 5-HT fibres may not. A single 5-HT fibre would influence all microzones and zones along its trajectory, inconsistent with the suggestion that 5-HT might serve as a responsibility signal to select a specific set of microzones sufficient to form the basis of an internal model for a particular movement (Schweighofer et al , Brain Res Rev 44:103,2004).
NA function in cerebellar consolidation was revealed by local application of propanolol, an antagonist of both β1- and β2-adrenoceptors (Paredes et al 2009). Here we report the distribution of both types. Immunofluorescent labelling of β1- and β2-adrenoceptor proteins clearly dissociated their cellular distribution in the cerebellar cortex. β1-adrenoceptor protein was entirely restricted to Purkinje neurons. In contrast, β2-adrenoceptor protein was restricted to the cell bodies and processes of Bergmann glia. Further studies with selective antagonists will reveal whether consolidation of cerebellar learning is β1 or β2-adrenoceptor-dependent and thus whether Purkinje cells or Bergman glia are directly implicated.

Disclosures: M. Longley: None. **C.H. Yeo:** None.

Poster

561. Cerebellum: Anatomy and In Vitro Studies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.03/BBB6

Topic: D.14. Cerebellum

Support: Alfred P Sloan Foundation Fellowship to ALP

Title: The cerebellar nucleocortical tract includes a corollary discharge pathway in mouse

Authors: *B. HOUCK[1], A. L. PERSON[2];
[1]Physiol. and Biophysics, Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [2]Physiol. and Biophysics, Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Theoretical models suggest that the cerebellum supports precise movements through feedforward motor control. Accurate feedforward computations are hypothesized to require information about outgoing motor commands via corollary discharge (CD) - a copy of an efferent motor command. A key candidate source of CD to the cerebellar cortex is the nucleocortical pathway. Previous studies in cats and primates have suggested that collaterals of nuclear output neurons may terminate in the cerebellar cortex, forming part of this pathway. These collaterals could provide a source of motor input to the cerebellum, but the literature is equivocal on its organization with recent data suggesting that the mouse nucleocortical pathway includes a glycinergic projection. To test whether the nucleocortical pathway in mice includes a corollary discharge pathway, we injected biotinylated dextran amines into the ventrolateral thalamus of wildtype mice, the targets of premotor nuclear output neurons. Following injections, we observed retrogradely labeled somata in the cerebellar nuclei and corresponding collateral terminals ascending into the granule cell layer making up the nucleocortical pathway (n = 8). Collaterals terminated as mossy fiber rosettes that were immunopositive for synaptic vesicle protein 2 (SV2). The most densely innervated cortical areas were the Simple, Crus I and Crus II lobules with a lighter terminal distribution within several other cerebellar lobules. Nucleocortical terminals formed close contacts with Golgi cells as visualized with antibody staining against neurogranin (n=4) and mGluR2/3 (n=2) or with genetic label using GlyT2-GFP transgenic mice (n=4), suggesting that this CD pathway could induce feedforward inhibition to modify convergent sensory pathways. To confirm that the mouse nucleocortical pathway also includes a glycinergic projection, we injected AAV-Syn1-mCherry into the cerebellar nuclei of transgenic GlyT2-GFP mice and traced double labeled neurons from the nucleus to their termination in the granule cell layer (n=6). These fibers did not terminate as mossy fibers but rather ended in simple boutonal swellings. These results (1) suggest a mechanism by which CD can modify sensory input through inhibitory interneurons and (2) confirmed the existence of an inhibitory nucleocortical population with direct input to the cerebellar cortex. These pathways could be critical for the computation of a forward model.

Disclosures: B. Houck: None. **A.L. Person:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.04/BBB7

Topic: D.14. Cerebellum

Support: CIHR (to DRW)

NSERC (to DRW)

Title: Parasagittal organization of visual mossy fiber projections from the nucleus lentiformis mesencephali to folium VIII of the pigeon cerebellum in relation to Zebrin II stripes

Authors: *J. P. LAM[1], D. J. GRAHAM[2], C. GUTIERREZ-IBANEZ[3], D. R. WYLIE[2]; [1]UBC, Vancouver, BC, Canada; [2]Dept. of Psychology, [3]Univ. Ctr. for Neurosci., Univ. of Alberta, Edmonton, AB, Canada

Abstract: The cerebellar cortex is organized into parasagittal zones. This has been shown with respect to the physiological responses of Purkinje cells, projections of Purkinje cells, afferent inputs from climbing and mossy fibres and the expression of several molecular markers including zebrin II (ZII; a.k.a. aldolase C). In numerous mammalian and avian species ZII is expressed heterogeneously in Purkinje cells, such that there are sagittal stripes of high expression (ZII+) interdigitated with stripes of little or no expression (ZII-). Several studies have attempted to determine how the ZII stripes are related to the other aforementioned aspects of cerebellar organization. For example, previously we have shown that the majority (~90%) of mossy fibres from the nucleus of the basal optic root (nBOR) and nucleus lentiformis mesencephali (LM) that project to the vestibulocerebellum terminate in sagittal bands that are aligned with the ZII+ stripes. The nBOR and LM are retino-recipient nuclei that process optic flow resulting from cell motion. The LM also projects to the folia VI-VIII in the posterior vermis. These folia also receive visual mossy fibre inputs from a tecto-potine involved in the analysis of local visual motion. This integration of optic flow with local-visual motion information is thought to be important from obstacle avoidance during locomotion through cluttered environments. In this study we examined the projection from LM to folium VIII in relation to the ZII stripes. Anterograde fluorescent tracer (BDA) was injected into LM, and serial sections through the posterior cerebellum were immunoprocessed for ZII expression. We found that, as is the case for the vestibulocerebellum, the majority (85-90%) of the mossy fibre rosettes terminated in sagittal bands that were aligned with the ZII+ve stripes.

Disclosures: J.P. Lam: None. **D.J. Graham:** None. **C. Gutierrez-Ibanez:** None. **D.R. Wylie:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.05/BBB8

Topic: D.14. Cerebellum

Support: CIHR (to DRW)

NSERC (to DRW)

NSERC (to ANI)

Title: Heterogeneity of calretinin expression in the avian cerebellar cortex of pigeons and relationship with zebrin II

Authors: *D. WYLIE[1], M. JENSEN[2], C. GUTIERREZ-IBANEZ[2], D. J. GRAHAM[1], A. N. IWANIUK[3];
[1]Dept. of Psychology, [2]Univ. Ctr. for Neurosci., Univ. of Alberta, Edmonton, AB, Canada;
[3]Canadian Ctr. for Behavioural Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: The cerebellar cortex has a fundamental parasagittal organization that is reflected in the physiological responses of Purkinje cells, projections of Purkinje cells, afferent inputs from climbing and mossy fibres inputs and the expression of several molecular markers. The most thoroughly studied of these molecular markers is zebrin II (ZII; a.k.a. aldolase C). ZII is differentially expressed in Purkinje cells, resulting in a pattern of sagittal stripes of high expression (ZII+) interdigitated with stripes of little or no expression (ZII-). The calcium binding protein calretinin (CR) is known to be expressed heavily in mossy fibres terminating throughout the cerebellar cortex although it is not known if it heterogeneously expressed with a parasagittal distribution. In this study we examined CR expression in the cerebellum of pigeons and compared it to that of ZII.
We found that CR was expressed heavily in the granule layer in mossy fibres and their terminal rosettes. Moreover, CR was expressed heterogeneously in the granule layer such that there were sagittal stripes of heavy CR labeling (CR+) alternating with stripes of weaker labeling (CR-). The CR heterogeneity was most notable in folium IXcd and folia II-IV in the anterior lobe. In the anterior lobe, the central CR+ stripe spanning the midline was aligned with the central ZII+ stripe, whereas the other CR+ stripes were aligned with the ZII-ve stripes. In IXcd, the CR+ stripes were aligned with the ZII+ stripes. We suggest that the CR+ mossy fibres to IXcd originate in two retinal recipient nuclei involved in the processing of optic flow.

Disclosures: D. Wylie: None. **M. Jensen:** None. **C. Gutierrez-Ibanez:** None. **D.J. Graham:** None. **A.N. Iwaniuk:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.06/BBB9

Topic: D.14. Cerebellum

Support: CONACyT: 377111

CONACyT: 418902

Promep: UVER-PTC-195 (LIGH)

Title: Olfactory stimulation by airborne scents induces c-Fos expression in the vermis cerebellum of sexually experienced male rats

Authors: P. GARCIA-BAÑUELOS, Z. HERNANDEZ-BRIONES, G. HERRERA-MEZA, G. ARANDA-ABREU, P. CARRILLO, M. HERNANDEZ, G. CORIA-AVILA, J. MANZO, *L. I. GARCIA;
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Abstract: The cerebellum functions have been ascribed related movements, reflexes, memory, sex, language, planning and forecasting, and perceptual ability. Recent studies have demonstrated that distance stimulation of male rats through sexual signals significantly increases the expression of c-Fos in the granular layer in each cerebellar lobe. Although this activity could correspond to sensory stimulation (visual, auditory or olfactory) or the motor execution.There is little information on the role of the cerebellum in sensory integration, particularly of smell The objective of the present study is to analyse and compare the expression of c-Fos protein in the cerebellar vermis male rats who have performed sexual behavior after being stimulated olfactory. Male rats (250-300 g) that have run one, three and five times the sexual behavior were placed alone for 5 min into the acrylic chamber (30 X 30 X 60 cm), and then 100 g of wood chip bedding was placed in a hide compartment underneath the floor, that had holes to prevent direct contact with it but allow olfactory stimulation. Test lasted 60 min with clean wood chip, wood chip impregnated with almond essence or wood chip from a bedding of receptive females. Finally, males were anaesthetized with an i.p. injection of sodium pentobarbital (60 mg/kg), a transcardial perfusion was made and immunohistochemistry done in the cerebellar tissue. Immunoreaction analysis of c-Fos protein in the granular layer of the cerebellar vermis shows an

increase. Olfactory stimulation showed an increase in the activity of the cerebellar vermis in the group stimulated with receptive female odor compared with almond and this in turn with the control. That pattern in all three groups increased with the acquisition of experience in sexual behavior. Our study confirmed that the cerebellum is activated differently to different stimuli and also that this activity is modified by acquired experience in sexual behavior.

Disclosures: P. Garcia-Bañuelos: None. **L.I. Garcia:** None. **Z. Hernandez-Briones:** None. **G. Herrera-Meza:** None. **G. Aranda-Abreu:** None. **P. Carrillo:** None. **M. Hernandez:** None. **G. Coria-Avila:** None. **J. Manzo:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.07/BBB10

Topic: D.14. Cerebellum

Support: KAKENHI No. 21220006

KAKENHI No. 22680031

KAKENHI No. 22650083

Title: Fine scale correspondence between the cerebellar microzones and the aldolase C compartments in mice

Authors: *S. TSUTSUMI[1], M. YAMAZAKI[2], K. SAKIMURA[2], K. KITAMURA[1], M. KANO[1];
[1]Univ. of Tokyo, Tokyo, Japan; [2]Niigata Univ., Niigata, Japan

Abstract: The cerebellar cortex has an elaborate zonal structure extending rostro-caudally. Purkinje cells (PCs) located in this parasagittal stripe show synchronous complex spike (CS) activities. This functional structure is considered to arise from anatomical organization of olivocerebellar projection (Sugihara et al., 2001) and electrical coupling among neurons in the inferior olive (Lang et al, 1999). The longitudinal compartment of aldolase C (zebrin II) expression in the cerebellar cortex has been shown to correspond to anatomical climbing fiber (CF) innervation from the inferior olive. Aldolase C compartments have been shown to correlate with functional complex spike synchrony at the resolution of several hundred micrometers (Sugihara et al., 2007). To elucidate the relationship between the CS synchrony and the aldolase C compartments at finer spatial resolution, we performed two-photon calcium imaging in

anesthetized mice that express fluorescent protein in their aldolase C-expressing PCs. We found a highly precise correlation between the CS synchrony and the aldolase C compartments. Spontaneous CS activities within aldolase C positive-PCs and those within aldolase C negative-PCs were both highly synchronized. The border of these high synchrony areas precisely corresponded to that of the aldolase C compartments at single cell resolution. We further investigated the relationship between the aldolase C compartments and functional microzones identified by responses to sensory stimuli. Fine microzonal structure could be activated in response to perioral air puff stimulation. The border of this sensory-evoked microzone precisely corresponded to that of aldolase C compartments. During awake state, sensory-evoked microzones could be extended and overlapped. These results suggest that aldolase C compartments represent physiological zones, which can cooperate during sensorimotor activities.

Disclosures: S. Tsutsumi: None. **M. Yamazaki:** None. **K. Sakimura:** None. **K. Kitamura:** None. **M. Kano:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.08/BBB11

Topic: D.14. Cerebellum

Support: NIH grant NS058500

NIH grant GM43459

National Ataxia Foundation Postdoctoral Fellowship

Title: Mapping cerebellar circuit function in zebrafish (Danio rerio) using electrophysiology and calcium imaging

Authors: J.-Y. HSIEH, F. A. ISSA, J. WAN, J. C. JEN, *D. M. PAPAZIAN;
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Abstract: The zebrafish, a lower vertebrate, provides numerous advantages for investigating the development of neuronal circuits and their role in controlling behavior, including rapid development outside the body of the mother, optical clarity early in life, and the ability to image neuronal activity in living animals using genetically-encoded calcium indicators (GECIs). However, GECIs have relatively low temporal resolution, limiting their usefulness for detailed analysis of circuit function. In addition, it is often difficult to identify the electrical events that

trigger calcium transients. In contrast, electrophysiological methods provide detailed information about neuronal activity but have not yet been widely used to investigate circuit function in the zebrafish brain. To characterize the development and maturation of electrical activity in the zebrafish cerebellum, we performed in situ patch clamp electrophysiology and in vivo calcium imaging in cerebellar Purkinje cells during the first 12 days post-fertilization (dpf). Patch clamp experiments were performed using an albino transgenic line that expresses a membrane-bound Venus fluorescent protein specifically in Purkinje cells under the control of the *aldoase Ca* (*aldoca*) promoter. Alternatively, a membrane bound GECI, lck-GCaMP5, was transiently expressed in Purkinje cells using the *aldoca* promoter. Spontaneous tonic firing and complex spikes were recorded using an extracellular patch electrode between 5-12 dpf. The frequency of tonic firing was ~10 Hz at room temperature (21-24^{o}C), whereas the frequency of complex spiking was between 0.2-0.5 Hz. Spontaneous calcium transients characterized by rapid rise and a decay phase that lasted several seconds were detected during the same time period. The development of a functional cerebellar circuit was monitored by direct electrical stimulation applied using a theta glass electrode. At 5 dpf, stimulation of the inferior olive evoked complex spikes, indicating that functional synapses had been made between climbing fibers and Purkinje cells. Previously, anatomical evidence has shown that cerebellar granule cells receive mossy fiber inputs from pretectal neurons in the anterior diencephalon. We investigated whether this pathway was functional. Stimulation of the pretectum increased the frequency of tonic firing in Purkinje cells and generated large calcium transients that sometimes spread to neighboring cells. Our results indicate that Purkinje cells, which are born beginning at 3 dpf in zebrafish, rapidly become electrically excitable and exhibit robust tonic firing and complex spiking as part of a functional cerebellar circuit by 5 dpf.

Disclosures: J. Hsieh: None. **D.M. Papazian:** None. **F.A. Issa:** None. **J. Wan:** None. **J.C. Jen:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.09/BBB12

Topic: D.14. Cerebellum

Support: National Health and Medical Research Council (NHMRC) Australia Fellowship Grant awarded to Professor George Paxinos (Grant #568605).

Title: Projections from the spinal cord to the cerebellar cortex in the mouse

Authors: *G. SENGUL[1], Y. FU[2,3], Y. YU[4], G. PAXINOS[2,3];
[1]Ege Univ. Sch. Med., Izmir, Turkey; [2]Neurosci. Res. Australia, Sydney, Australia; [3]The Univ. of New South Wales, Sydney, Australia; [4]Dept. of Pediatric Surgery, The First Affiliated Hosp. of Harbin Med. Univ., Harbin, China

Abstract: We have investigated the topography of projections from the spinal cord to the cerebellum using neuronal tracers Fluoro-Gold (FG) and cholera toxin B (CTB). Although there are a number of studies on spinocerebellar projections, the majority concern the connections of cerebellar nuclei and, hitherto, there is no detailed study on the distribution of spinal cord neurons projecting to the cerebellar cortex, either vermis or hemispheres. Pressure injections of FG or CTB were made into the lobules 1-10 of the cerebellar vermis (1Cb to 10Cb) and on the right side of the cerebellar lobules (simple lobule, flocculus, paraflocculus, paramedian lobule, copula of the pyramis, and Crus1 and 2 of the ansiform lobule) on 13 C57BL/6J mice. Projections to 1Cb, 2Cb and 3Cb of the vermis were found from the central cervical nucleus (CeCv), deep dorsal horn (DDH), dorsal nucleus (D), lumbar and sacral precerebellar nuclei (LPrCb and SPrCb, respectively), and laminae 7-8 and 10 neurons. We did not find any neurons projecting to 4-7Cb. The 8Cb and 10Cb received projections from the CeCv, DDH, lamina 7, LPrCb and SPrCb neurons, while the crus1 and crus2 of the ansiform lobule received afferents from the DDH and laminae 7-8 neurons. The paraflocculus and flocculus received projections from neurons of the intermediolateral nucleus (IML), lamina 10 (presumably paraependymal part of the intercalated nucleus) and the lumbar dorsal commissural nucleus. Projections to the copula of the pyramis were from the DDH (mainly lamina 5), laminae 7, 10, D and LPrCb. The distribution of spinocerebellar neurons were mostly bilateral; however, in some cases, CeCv neurons were more numerous contralateral to the site of the cerebellar injection, and DDH neurons were more numerous ipsilaterally.

Disclosures: G. Sengul: None. **Y. Fu:** None. **Y. Yu:** None. **G. Paxinos:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.10/BBB13

Topic: D.14. Cerebellum

Support: National Research Foundation of Korea (NRF) Grant 2009-0080939

National Research Foundation of Korea (NRF) Grant 2011-0030737

Title: Lower level of inhibitory synaptic transmission in the vestibulo cerebellum

Authors: C. RYU, J. KIM, *S. KIM;
Physiol., Seoul Natl. Univ. Coll Med., Seoul, Korea, Republic of

Abstract: In accordance with functional classification, the cerebellum is categorized as three subdivisions, the cerebro-cerebellum, the spino-cerebellum, and the vestibulo-cerebellum (VC). It has been known that the VC, or flocculonodular lobe which is comprised of flocculus, paraflocculus, lobule IX and X of vermis receives substantial vestibular input directly from vestibular nuclei, and its extensive output projects to vestibular nuclei. Among several types of neurons in the cerebellar cortex, Purkinje cells (PCs) on which external inputs via parallel fibers and climbing fibers converge are the exclusive output neurons and, accordingly, regarded as the principal neurons of the cerebellar circuits. Therefore, understanding properties of PCs that are related to input-output is necessary for comprehending the operating principles of the cerebellar circuitries. With distinguishable characteristics of input and output functions in the vestibule-cerebellum, previous studies have shown that PCs in the VC have distinct electro-physiological properties. For example, PCs have higher resistance to excitatory synaptic plasticity and lower intrinsic excitability in the VC than those in any other cerebellar regions. Besides excitatory inputs via parallel fibers and climbing fibers, PCs receive inhibitory inputs from molecular layer interneurons which form feed-forward inhibition influencing the fidelity of PCs output. However, there has been no research regarding inhibitory synaptic transmission of the VC. In this study, we investigated the nature of inhibitory synaptic transmission in the VC and their functional implication, using whole cell patch clamp techniques. Since cerebellar vermis, which is subdivided into ten lobules, has both the VC (lobule IX and X) and the spino-cerebellum (the other lobules), we compared PCs between lobules III~VI and lobule X of vermis. We found that inhibitory inputs into PCs of the VC have much lower amplitude, but not lower frequency than those in other cerebellar regions. In an aspect of the relationship between amplitude and rise time, it is plausible that this difference is due to basket cell-PC synapse rather than stellate cell-PC synapse. In addition to the variation of basal inhibitory level, PCs of lobule X showed limited inhibitory synaptic plasticity such as rebound potentiation or DSI. Paradoxically, although inhibitory inputs are much lower in the VC, their effect on the output of PCs (Spontaneous Firing of PCs) is more profound in the VC. Thus, we suggest that the lower level of inhibitory synaptic transmission in the VC enables the output of PCs to be regulated by subtly altering the inhibition pattern.

Disclosures: C. Ryu: None. **J. Kim:** None. **S. Kim:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.11/BBB14

Topic: D.14. Cerebellum

Support: NIH Grant R01 DC004450

NIH Grant F31 DC013223

Title: A granule cell - golgi cell feedback circuit in the cochlear nucleus

Authors: *D. B. YAEGER[1], L. O. TRUSSELL[2];
[1]Physiol. and Pharmacol., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Oregon Hearing Res. Ctr. / Vollum Inst., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The dorsal cochlear nucleus (DCN) is a cerebellum-like circuit positioned at the earliest stage of auditory processing in the brainstem. Auditory granule cells receive excitatory nonauditory input from mossy fibers. Parallel fibers are the axons of granule cells and project to DCN principal cells. Granule cells are subject to prominent inhibition, but the source of the inhibition is unknown. Using paired recordings in mouse brain slices (P16-24), we identified GABAergic, mGluR2-positive Golgi cells as at least one local source of inhibition to granule cells. Moreover, granule cells frequently made monosynaptic excitatory, facilitating synapses onto Golgi cells. Indeed, approximately 25% of connected granule-to-Golgi cell pairs were also connected in the Golgi-to-granule cell direction. Thus, feedback inhibition is a prominent feature of this circuit. However, most unitary granule-to-Golgi cell synapses were too weak to excite Golgi cells, indicating that multiple granule cells are required to drive Golgi cells. To monitor feedback inhibition, we therefore used extracellular stimulation of groups of parallel fibers in the DCN molecular layer to synaptically activate Golgi cells while recording from either Golgi or granule cells. Golgi cells were most effectively excited when stimulating parallel fibers at high frequencies, as expected due to the strong facilitation of parallel fiber synapses. Similarly, feedback inhibition onto granule cells also showed facilitation and was best recruited when stimulating parallel fibers at high frequencies. Facilitation of inhibition was surprising because Golgi-to-granule cell synapses exhibited prominent synaptic depression when Golgi cells were directly stimulated. The build-up of Golgi cell firing probability during parallel fiber stimulation may therefore mitigate depression of feedback inhibition. Our results suggest that feedback inhibition modifies information transfer between granule cells and their postsynaptic targets by selecting for particular patterns of mossy fiber activity.

Disclosures: D.B. Yaeger: None. **L.O. Trussell:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.12/BBB15

Topic: D.14. Cerebellum

Support: CEREBNET PITNGA- 2009-238686

Edmond and Lily Safra Centerfor Brain Sciences grant

Title: Cerebellar control of electronic coupling in the inferior olive I: Anatomy of the nucleo-olivary projection

Authors: *M. Y. UUSISAARI[1], Y. LEFLER[2], Y. YAROM[2];
[1]Optical Neuroimaging Unit, [2]Hebrew Univ. of Jerusalem, Israel, Jerusalem, Israel

Abstract: The olivo-cerebellar loop consists of cerebellar cortex, the cerebellar nuclei and the inferior olive (IO). The GABAergic projection from the cerebellar nuclei to the inferior olive (the nucleo-olivary (NO) pathway) has long been considered a critical part of the olivo-cerebellar circuitry and knowledge of its properties is critical for formulation of conceptual and computational models of the cerebellar function.

Specifically, it has been proposed that the cerebellar GABAergic input to the IO acts mainly via a shunting mechanism, reducing the coupling coefficient between the IO neurons. However, this proposed functionality has not been experimentally demonstrated.

In the present two works we employ targeted expression of optogenetic tools to I) examine the anatomical organization of this pathway and II) specifically activate the GABAergic nucleo-olivary axons in a spatially constrained manner.

In the first (anatomical) part of the study, we utilize single- and double labeling of the neuronal circuit elements (NO and IO neurons) by viral transfection as well as by intracellular filling of individual IO neurons via patch electrode. This allows us to create 3D reconstructions of the nucleo-olivary circuit structure. Examining the olivary volume thus labeled demonstrates that the extremely dense GABAergic innervation of the IO is mostly targeting dendritic locations. Furthermore, we explore the regional variation of the cerebello-olivary projection, prompting for further examinations of the components of this structure.

Disclosures: M.Y. Uusisaari: None. **Y. Lefler:** None. **Y. Yarom:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.13/BBB16

Topic: D.14. Cerebellum

Support: ISF- Israeli Science Foundation

FP7 people initial training network grant (CEREBNET)

CEREBNET PITNGA 2009-238686

FP7-ICT (REALNET)

Edmond and Lily Safra Center for Brain Sciences (ELSC)

Title: Cerebellar control of electronic coupling in the inferior olive II: Optogenetic activation of the nucleo-olivary pathway modulates coupling coefficient and sub-threshold oscillations

Authors: *Y. LEFLER, M. Y. UUSISAARI, Y. YAROM;
Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The olivo-cerebellar loop consists of cerebellar cortex, the cerebellar nuclei and the inferior olive. The GABAergic projection from the cerebellar nuclei to the inferior olive has long been considered a critical part of the olivo-cerebellar circuitry and knowledge of its properties is critical for formulation of conceptual and computational models of the cerebellar function. Specifically, it has been proposed that the cerebellar GABAergic input to the inferior olive acts mainly via a shunting mechanism, reducing the coupling coefficients between inferior olive neurons. However, regardless of the importance of this pathway in the cerebellar network, due to technical limitations this proposed functionality has not been experimented.
In the present work we virally expressed ChR2 in GABAergic cells of the cerebellar nuclei and specifically activate the GABAergic nucleo-olivary axons in a spatially constrained manner in the inferior olive in-vitro. Using patch clamp recordings of inferior olive cells we show for the first time that GABA release from nucleo-olivary terminals has 3 significant effects: 1. reducing the coupling coefficient between pairs of olivary neurons. 2. Changing the asymmetry of the coupling 3. Completely eliminates the spontaneous subthreshold voltage oscillations of olivary neurons. These effects have substantial consequences on our understanding of the functional organization in the olivo-cerebellar system.

Disclosures: Y. Lefler: None. **M.Y. Uusisaari:** None. **Y. Yarom:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.14/BBB17

Topic: D.14. Cerebellum

Support: CEREBNET PITNGA- 2009-238686

Edmond and Lily Safra Centerfor Brain Sciences grant

Title: Visualization of the Purkinje neuron to cerebellar nuclei anatomical connectivity using a viral approach

Authors: *H. NEDELESCU[1,2], G. ARBUTHNOTT[1], B. KUHN[1], E. DE SCHUTTER[1,2], Y. YAROM[3], M. Y. UUSISAARI[1,3];
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Abstract: The cerebellar nuclei (CN) play an important role in the temporal and spatial control of motor function. Despite the fact that the CN integrate information from the entire cerebellar cortex via axonal projections from the inhibitory Purkinje neurons (PNs), the anatomical organization of the PN to CN connectivity has been difficult to address due to technical limitation. In order to determine the manner in which information is being processed between the PNs and their target nuclei, we designed a viral transfection technique in which we achieved a differential expression of fluorescent reporters in CN neurons and PNs of adult mice. The resulting non-overlapping fluorescent expression pattern allowed confocal imaging and unambiguous reconstruction of both CN neurons and PN axons. This enabled the comparison of the varied PN innervation pattern among a population of different CN neurons. Examining the ratio of PN terminals innervating the CN soma versus dendrites demonstrated that at the population level most of the reconstructed CN cells had stronger PN innervation on the soma than on the dendrites; however, in a subpopulation of CN neurons the PN innervation was denser on the dendrites. The observed differences of connectivity pattern of PNs to CN point towards different readouts indicative of different PN activity input to the CN neurons, and call for further exploration of the CN neuronal diversity.

Disclosures: H. Nedelescu: None. **G. Arbuthnott:** None. **B. Kuhn:** None. **E. De Schutter:** None. **M.Y. Uusisaari:** None. **Y. Yarom:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.15/BBB18

Topic: D.14. Cerebellum

Support: NIH Grant R01 MH56661

NIH Grant R01 NS24328

NIH Grant P40 OD010996

NIH Grant P30 NS076405

Title: Interconnections between the lateral cerebellum and the prefrontal cortex

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Abstract: Kelly and Strick (‘03) proposed that cerebro-cerebellar connections are organized as closed-loop circuits. They demonstrated this structural arrangement for a motor circuit with the arm area of the primary motor cortex, and for a non-motor circuit with area 46 of the prefrontal cortex. In particular, area 46 sends projections to Crus II in the lateral cerebellar cortex and is a target of output from Crus II. Several other areas in the prefrontal cortex send inputs to the cerebellar cortex via the pons (Glickstein et al. ‘85) and are known targets of outputs from the lateral cerebellum (Middleton & Strick ’01). Here, we further explored how the connections between the lateral cerebellum and regions of the prefrontal cortex are organized at the level of the cerebellar cortex. We used retrograde transneuronal transport of rabies virus (RV) to investigate whether prefrontal areas that provide dense inputs to Crus II are also targets of output from Crus II. We injected RV into Crus II of cebus monkeys (n=3) and set the survival time at 42 hours to allow retrograde transport of RV to first-order neurons that project to the cerebellar cortex and then, retrograde transneuronal transport of RV from these neurons to second-order neurons in the neocortex. We observed RV labeling in several non-motor neocortical areas, with substantial numbers of labeled neurons in the rostral dorsal premotor cortex (pre-PMd) and lateral area 9 (9l) of the prefrontal cortex. We then injected RV into pre-PMd (n=2) and 9l (n=1) to determine whether these regions receive projections from Crus II. We set the survival time to 60 hours to allow retrograde transport of RV to first-order neurons in the thalamus that project to the cerebral cortex, retrograde transneuronal transport of RV from these neurons to second-order neurons in the cerebellar nuclei, and retrograde transneuronal transport of RV from the cerebellar nuclei to third-order neurons (Purkinje cells) in the cerebellar cortex. After injections of RV into either the pre-PMd or 9l, we observed large numbers of Purkinje cells labeled in Crus II. These results further support the hypothesis that prefrontal regions with dense inputs to Crus II are also targets of Crus II output. Interestingly, we observed potential regions of overlap between the areas in Crus II that send projections to pre-PMd, 9l, and area 46. This finding raises the question of whether the closed-loop circuits linking Crus II with the prefrontal cortex are distinct or

partially overlapping. Future experiments using dual labeling techniques will be necessary to answer this question and clarify the topography of connections between the lateral cerebellum and the prefrontal cortex.

Disclosures: A.C. Bostan: None. **R.P. Dum:** None. **P.L. Strick:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.16/BBB19

Topic: D.14. Cerebellum

Support: CEREBNET PITN-GA-2009-238686

Edmond and Lili Safra Center for Brain Science grants

Title: Projections from the cerebellar nuclei to the cerebellar cortex: A possible feedback to golgi cells in the cerebello-olivary loop

Authors: *L. ANKRI, Y. YAROM, M. Y. UUSISAARI;
Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The cerebello-olivary feedback loop consists of an inhibitory connection originating from Purkinje neurons (PN) in the cerebellar cortex to the cerebellar nuclei (CN), followed by a projection to the inferior olive by inhibitory CN neurons. This pathway is considered a critical part of the cerebellar circuitry that is thought to control PN spiking via modulating inferior olivary activity. Additionally, there is anatomical evidence for an opposite synaptic pathway (the nucleo-cortical), directly connecting the CN to the cerebellar cortex (Tolbert et al., 1976). However, this pathway and the implied possibility of direct feedback from CN to the cerebellar cortex have been mostly ignored in physiological studies.
Notably, the anatomical evidence for nucleo-cortical pathway has not identified the postsynaptic targets in the cerebellar cortex. In this work we set to clarify this with electrophysiological recordings from adult mouse cerebellum slices. Electrical stimulation of the white matter between the CN and cerebellar cortex evoked inhibitory synaptic responses in Golgi cells, suggesting that they are among the targets of nucleo-cortical neurons. In addition to the electrophysiological experiments, we used specific viral transfections to anatomically examine the connection. We suggest that the main elements for this proposed connection are the glycinergic projection neurons of the lateral CN ("Gly-I"; Uusisaari & Knöpfel, 2010). Interestingly, unlike all other CN neurons, the Gly-I neurons are not spontaneously active but

show very short and fast bursts of spikes in response to synaptic input, possibly implying a role in controlling timing in the granule cell layer.
Tolbert DL, Bantli H, Bloedel JR (1976) Anatomical and physiological evidence for a cerebellar nucleo-cortical projection in the cat. Neuroscience 1:205-217
Uusisaari M, Knöpfel M (2010) GlyT2+ Neurons in the Lateral Cerebellar Nucleus. Cerebellum. March; 9(1): 42-55.

Disclosures: L. Ankri: None. **Y. Yarom:** None. **M.Y. Uusisaari:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.17/BBB20

Topic: D.14. Cerebellum

Support: NIH P40 ODO10996

NIH RO1 MH084016

Title: The cytoarchitecture and neurochemical profile of the rat deep cerebellar nuclei with a focus upon nucleus interpositus

Authors: *J. P. CARD[1], D. W. VOLK[2], E. J. SENGUPTA[2], N. Z. KHAN[3], G. J. WOJACZYNSKI[3];
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Abstract: The deep cerebellar nuclei (DCN) are complex computation units that integrate afferents from the cerebellar cortex and collaterals of axons entering the cerebellar hemispheres. Whereas the synaptic and functional organization of the cerebellar cortex is well described, DCN organizational principles are only generally understood, and it is not know if they differ among the deep nuclei. Data from a variety of experimental approaches suggest that functional localization within the cerebellar cortex is also represented in the DCN. For example, the dorsolateral portion of the anterior interpositus nucleus is known to mediate classical conditioning of the eyeblink reflex. Here we test the hypothesis that the DCN contain subfields distinguished by cytoarchitecture and the differential distribution of phenotypically defined neurons. In the first stage of the analysis the brain of an adult male Sprague-Dawley rat was paraffin embedded and sectioned serially at 12 μm/section. Sections at a frequency of 60 μm were stained with the Klüver-Barrera technique and subjected to detailed cytoarchitectural analysis. Distinct subfields in each of the DCN were distinguished by neuronal morphology,

cellular packing density, and the extent of neuropil. Morphologically distinct classes of neurons included 1) large, intensely basophilic, multipolar neurons ranging in size from 35 to 70 μm in widest diameter; 2) intensely basophilic neurons ranging from 25 to 35 μm in diameter; 3) small intensely basophilic neurons with a diameter less than 25 μm; and 4) small pale staining neurons less than 25 μm in diameter. In situ hybridization characterization of neuronal phenotype using a radiolabeled riboprobe generated against the glycine transporter 2 (GlyT2) supported the conclusion that cytoarchitecturally distinct classes of neurons were also distinguished by neurotransmitter phenotype. For example, GlyT2 mRNA+ neurons in nucleus interpositus were of intermediate size and distributed through all cytoarchitecturally distinct subfields, whereas GlyT2 mRNA+ neurons in the fastigial nucleus constituted two populations of neurons: large cells differentially concentrated in the ventral portion of the nucleus and neurons of intermediate size distributed throughout the nucleus. Ongoing studies using riboprobes generated against GAD65, GAD67 and vGlut1 mRNAs will provide further insight into the hypothesis that subfields of the DCN defined by cytoarchitecture can be further subdivided on the basis of transmitter phenotype. Collectively the data are consistent with a modular organization of the DCN and provide a strong foundation for defining the synaptology of each subfield.

Disclosures: J.P. Card: None. **D.W. Volk:** None. **E.J. Sengupta:** None. **N.Z. Khan:** None. **G.J. Wojaczynski:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.18/BBB21

Topic: D.14. Cerebellum

Support: NINDS/NIH (R01 NS040863)

The Hand Embodied (THE), EU FP7, project no. 248587

Swedish Research Council (VR Medicine)

Title: Model of interactions between spinal border cells and cerebellar granule cells

Authors: *A. SPANNE, P. GEBOREK, F. BENGTSSON, H. JÖRNTELL;
Lund Univ., Lund, Sweden

Abstract: Spinocerebellar systems are likely to be crucial for cerebellar hallmark functions such as coordination. However, in terms of cerebellar functional analyses, these are perhaps among

the least explored systems. The aim of the present study is to achieve activation of a single component of the spinocerebellar systems and to explore to what extent it can influence the spike output of granule cells. For this purpose, we took advantage of a unique arrangement discovered in neuroanatomical studies, in which the spinal border cell component of the ventral spinocerebellar system was found to be the only the only spinocerebellar tract which ascends in the contralateral lateral funiculus and have terminations in sublobulus C1 of the paramedian lobule in the posterior cerebellum. Using electrical stimulation of this tract, we found that a subset of the granule cells displayed high intensity responses whereas the majority of the granule cells displayed no response at all. A model of the spinocerebellar interactions was constructed, in which the integration of motor command signal and sensory feedback by SBC neurons during motor control was simulated using behavioral data. The model illustrates how this information becomes represented at the level of the granule cells. The implications of these findings for the view of the cerebellar cortex as a linear integration circuitry for multi-dimensional sensorimotor signals (Spanne and Jörntell (2013) PLoS Comp Biol) are discussed.

Disclosures: A. Spanne: None. **P. Geborek:** None. **H. Jörntell:** None. **F. Bengtsson:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.19/BBB22

Topic: D.14. Cerebellum

Support: RIKEN Strategic Program for R&D

Title: NanoCAGE analysis of neuronal translatome with subcellular resolution

Authors: *T. LAUNEY[1], A. KRATZ[2], P. BEGUIN[1], M. KANEKO[1], T. CHIMURA[1], A. SUZUKI[2], N. BERTIN[2], R. VIGOT[1], P. CARNINCI[2], C. PLESSY[2];
[1]Brain Sci. Inst. (RIKEN), Wako-Shi, Japan; [2]Ctr. for Life Sci. Technologies, RIKEN, Yokohama, Japan

Abstract: Development and synaptic plasticity in complex neurons requires local protein synthesis. To clarify the mechanisms controlling protein synthesis in the Purkinje cell and in particular in its dendrites, we combined several technologies to specifically extract ribosomes from this neuron. As the main component of the protein synthesis machinery, the ribosome is transiently bound to the messenger RNAs being translated into proteins. By encoding a ribosome-binding probe (“TRAP”) into a virus vector targeted to PCs, we succeeded to capture several thousand ribosome-bound PC RNAs (translatome). Differential fractionation allowed us

to discriminate cytoplasmic polysome from ER-associated ribosomes. High-sensitivity NanoCAGE and deep-sequencing were then used to identify all the isolated RNAs, either as protein-coding sequences or as regulatory RNAs. Using microdissections to separate the PC dendrites from its cell body, we could identify a group of several hundred RNAs that appear to be transported into dendrites for remote translation. Interestingly, in addition to transcripts of protein known to be involved in synaptic plasticity, we also found that these ribosome-bound RNAs include a large fraction of transcripts with no known function, representing either novel genes or regulatory RNA sequences.

Disclosures: T. Launey: None. **A. Kratz:** None. **M. Kaneko:** None. **P. Beguin:** None. **T. Chimura:** None. **A. Suzuki:** None. **N. Bertin:** None. **P. Carninci:** None. **R. Vigot:** None. **C. Plessy:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.20/BBB23

Topic: D.14. Cerebellum

Support: NIH Grant AA018747

Title: A novel transgenic model for protecting motoric function from the repeated use of benzodiazepine

Authors: *M. E. JUNG, D. B. METZGER;
Univ. N Texas Hlth. Sci. Ctr., FORT WORTH, TX

Abstract: Purpose: Benzodiazepine (BZD) is the most prescribed CNS depressant in America to treat hyper-excitatory disorders such as anxiety and insomnia. However, a prolonged use of BZD often creates adverse effects including psychomotor deficit. In this study, we investigated a novel protective mechanism by which chronic BZD-induced motoric deficit can be minimized in mice. We tested the hypothesis that the down-regulation of p38 (stress-activated protein) in cerebellar Purkinje neurons contributes to protection for motoric function from chronic BZD use.
Methods: transgenic mice that were designed to lack p38 in cerebellar Purkinje neurons were generated by crossing Pcp2 (Purkinje cell protein 2)-Cre mice with p38loxP/loxP mice. When they reached 21 days old, genotyping was conducted using PCR. BZD injection (lorazepam, 0.5 mg/kg, IP) began when mice were two months old. Wild-type (C57BL/6 mice) or the transgenic mice lacking Purkinje p38 received BZD in the afternoon, and next morning they were tested on Rotarod in which a quicker fall from rotating rod indicates poorer motoric performance. This

procedure was repeated for 14 days. Cerebellum was then collected to detect p38 in Purkinje neurons and to measure mitochondrial respiration using immunohistochemistry and real-time XF respirometry, respectively.
Results: Compared to vehicle-treated mice, BZD-treated mice showed poorer motoric performance, a higher number of Purkinje neurons containing p38, and lower mitochondrial respiration. These effects of BZD were much smaller in p38-knockdown mice.
Conclusions: These results suggest that the excessive accumulation of p38 in cerebellar Purkinje neurons contributes to motoric deficit and mitochondrial respiratory inhibition associated with chronic BZD. Our findings may provide a new mechanistic insight into chronic BZD-induced motoric deficit.

Disclosures: M.E. Jung: None. **D.B. Metzger:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.21/BBB24

Topic: D.14. Cerebellum

Support: CEREBNET FP7 ITN238686

REALNET FP7 ICT270434

The Italian Ministry of Health RF2009

the Italian Ministry of Health GR-2009-1493804

Title: The mechanisms of late-onset synaptic responses in a realistic model of Unipolar Brush Cells

Authors: S. SUBRAMANIYAM[1,2], F. LOCATELLI[1], P. PERIN[1], S. MASETTO[1], S. SOLINAS[3], *E. D'ANGELO[1,3];
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Abstract: Unipolar brush cells (UBCs) are excitatory glutamatergic interneurons of the cerebellar granular layer receiving both primary and secondary vestibular inputs through mossy fibers (excitatory input) and Golgi cell axon (inhibitory input). When injected with a depolarizing current, the UBC generates a spike burst sustained by a low-threshold spike, which,

at higher current intensity, is followed by a tonic firing showing frequency adaption. The intrinsic excitability of UBCs is characterized by an H current and by Low Voltage activated and High Voltage activated calcium currents. Fast inactivating T-type Calcium channels generate the low-threshold spikes while L-type Calcium channel sustain tonic firing. The H current (activating in the hyperpolarizing direction) produces a slow hyperpolarization characterized by a ”sag” and a rebound depolarization at the end of a depolarizing step. Here we present a biologically realistic multi-compartmental mathematical model of the UBC realized with the NEURON simulator. According to experimental data, the model includes 9 different ionic channels generating voltage-dependent A-type M-type BK-type and DR-type K currents, Na (transient, persistent and resurgent) currents, T-type and L-type Ca currents, the H-current and the TRP-current. These channels are sorted and distributed among compartments (soma, dendrite, and axon). The UBC model faithfully reproduces the excitable properties of real UBCs. In particular, the model includes mechanisms capable of reproducing the recently characterised "late onset response" (Locatelli et al., 2013). These mechanisms are based on the modulation of TRP- and Ih-channels by the activation of G-protein-dependent intracellular pathways yielding to the increase of cAMP concentration. This model, in addition to confirm the primary role of the aforementioned currents in UBC's electroresponsiveness, will prove a valuable tool for investigating the UBC's function in the cerebellar network.

Disclosures: S. Subramaniyam: None. **E. D'Angelo:** None. **F. Locatelli:** None. **P. Perin:** None. **S. Masetto:** None. **S. Solinas:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.22/BBB25

Topic: D.14. Cerebellum

Support: NIH Grant R01 DC004450

NIH Grant F31 DC012454

Title: Glutamate-activated currents in unipolar brush cells

Authors: *C. GLOGOWSKI[1], L. TRUSSELL[2];
[1]Vollum Inst. - Neurosci. Grad. Program, [2]Oregon Hearing Res. Ctr. / Vollum Inst., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Unipolar brush cells (UBCs) of the dorsal cochlear nucleus (DCN) and vestibular cerebellar cortex receive glutamatergic mossy fiber input on a complex brush-like dendrite. Studies of cerebellar UBCs identified different subtypes of UBC based on immunohistochemical markers and physiological profiles. We have examined auditory and cerebellar UBCs with respect to the physiology of the mossy input and glutamate sensitivity. Patch-clamp recordings were made in brain slices from P16-30 mice. Extracellular stimulation evoked biphasic NBQX-sensitive EPSCs with a profile similar to that reported in cerebellum: a small, fast inward current followed by a very slow delayed inward current. However, in some cells a train of stimuli would result in a delayed outward current consistent with a glutamate-activated K^+ current. We determined the glutamate receptors expressed in UBCs by applying 1 mM glutamate as well as selective agonists and antagonists. A variety of response profiles were elicited, suggestive of different levels of expression of glutamate receptors and two different UBC subtypes. UBC subtype 1 had a biphasic inward current, which is primarily AMPAR mediated, with a small mGluR1α component. The biphasic AMPAR component is similar to the synaptically evoked current and strongly excited UBCs. In this cell type, mGluR2 mediated outward currents were extremely weak, and were thus overwhelmed by AMPAR-mediated currents. In contrast, UBC subtype 2 responded to glutamate with a prominent outward current, occasionally preceded by a small inward transient mediated by AMPAR. The mGluR2 mediated outward K^+ current caused a prolonged hyperpolarization of membrane potential and pause in action potential firing. The pause was followed by rebound high-frequency firing mediated by a hyperpolarization-activated current and voltage-activated Ca^{2+} channels. This subtype was mGluR1α negative. In summary, subtype 1 UBC had an excitatory response to glutamate, due to high expression of AMPARs and mGluR1α, and low expression of mGluR2. Subtype 2 UBC had an inhibitory response to glutamate due to a low expression of AMPARs and high expression of mGluR2 and GIRK channels. These UBC subtypes may function as ON and OFF cells in regard to their response to excitatory input, and may thus provide distinct processing of multisensory input to their targets.

Disclosures: C. Glogowski: None. **L. Trussell:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.23/BBB26

Topic: D.14. Cerebellum

Support: NIH NS09904

Title: The TRPC3 moonwalker gain-of function mutation is accompanied by selective and complete ablation of type II unipolar brush cells

Authors: *J.-A. KIM[1], G. SEKERKOVÁ[2], E. B. E. BECKER[3], J. HARTMANN[4], L. BIRNBAUMER[5], E. MUGNAINI[2], M. MARTINA[1];
[1]department of physiology, [2]Dept. of Cell. and Mol. biology, Northwestern University, Feinberg Sch. of Med., Chicago, IL; [3]Univ. of Oxford, Oxford, United Kingdom; [4]Tech. Univ. of Munich, Munich, Germany; [5]NIEHS, Research Triangle Park, NC

Abstract: Unipolar brush cells (UBCs) are glutamatergic interneurons in the cerebellar granule cell layer, which are enriched in the vestibulocerebellum. UBCs receive monosynaptic input from external mossy fibers, mainly the primary and secondary vestibular afferents and transfer signals to a myriad of granule cells through their axonal terminals (intrinsic mossy fiber). Thus, they are suggested to act as an amplifier of the vestibular signal. Recently data show that UBC can be divided into two histochemically and functionally different subtypes; type I and type II. In situ hybridization suggests that the UBCs may express TRPC3 (Schilling and Oberdick, 2009). In this study we investigated whether TRPC3 are indeed present in UBC. To this end we used immunohistochemistry and patch clamp recording from cerebella of wild-type and TRPC3-/- mice. Using UBC type specific markers mGluR1α (type I) and calretinin (type II) we show that TRPC3 channel are expressed selectively in type II UBCs. Moreover we show that activation of group I mGluR in type II UBCs gates TRPC3 channels. In moonwalker (MWK/+) mice, an ataxia model, which have a heterozygous dominant gain-of-function TRPC3 mutation, type II UBCs were totally ablated from 18 day-old when their ataxic behavior is obvious. Depletion of type II UBCs may contribute to the ataxic phenotypes of MWK/+ mice. We suggest that fine regulation of calcium influx through TRPC3 plays an important role in neuronal cell survival, especially, in type II UBCs, where no major calcium binding proteins have been identified so far.

Disclosures: J. Kim: None. **G. Sekerková:** None. **E. Mugnaini:** None. **M. Martina:** None. **E.B.E. Becker:** None. **J. Hartmann:** None. **L. Birnbaumer:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.24/CCC1

Topic: D.14. Cerebellum

Support: NIH NS09904

Title: Early electrophysiological impairment of cerebellar Purkinje cells in the moonwalker mouse

Authors: *M. J. NIGRO, G. SEKERKOVA, E. MUGNAINI, M. MARTINA;
Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: The moonwalker mouse is a recently described rodent model of ataxia. This mouse carries a gain-of-function mutation of the gene encoding for the TRPC3 channel; this mutation leads to increased mGLUR1 receptor-dependent activation of the channel and possibly to a constitutively open channel (Becker et al., 2009). The moonwalker mouse shows severe motor deficits such as gait abnormalities, retropulsion and impaired balance as early as 3 postnatal weeks of age. Additionally, moonwalker mice show altered dendritic development of Purkinje cells and a progressive loss of these neurons. Such morphological changes however only become apparent around 4 months of age and more pronounced from 6 months of age. Little is known about the mechanisms that account for the early development of the ataxia, which precedes the morphological changes by several months. The mismatch between the onset of the motor deficits and the loss of Purkinje cells suggests that different mechanisms might be involved in the generation of the phenotype. In the present work we study the intrinsic properties of Purkinje cells in moonwalker mice. Our results show that, already at three weeks of age, Purkinje cells of moonwalker mice, although normal in gross morphological features, show severe electrophysiological alterations. In particular, cell-attached recordings in acute slices from moonwalker mice showed that only ~10% of these Purkinje cells were spontaneously active, while all cells from control animals were spontaneously firing. Moreover, whole cell recordings showed a reduced excitability in response to depolarizing current steps, and a largely reduced voltage sag in response to hyperpolarizing current steps, suggesting a downregulation of HCN channels in these animals. These results show that intrinsic properties of Purkinje cells of moonwalker mice are severely affected at a very early developmental stage and suggest that these functional changes may contribute to the ataxic phenotype.

Disclosures: M.J. Nigro: None. **G. Sekerkova:** None. **E. Mugnaini:** None. **M. Martina:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.25/CCC2

Topic: D.14. Cerebellum

Support: HHMI

NIH Grant EY-11027

Title: Classification of fastigial/medial cerebellar nucleus neurons by quantitative single-cell gene expression profiling

Authors: *H. FUJITA, T. KODAMA, S. GUERRERO, S. DU LAC;
The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Increasing evidence indicates that medial part of the cerebellum plays an important role not only in body posture and eye movements, but also in controlling physiological homeostasis, autonomic regulation, cognitive clarity, and emotional learning. However, the medial cerebellar (fastigial) nucleus (MCN), the sole output from the medial cerebellum, comprises multiple functional cell types that are spatially intermingled, making circuit studies challenging. To functionally classify MCN neurons and identify molecular handles for manipulating each cell type, we quantitatively profiled expression of genes related to neurotransmitters, ion channels, and marker gene candidates for individual MCN neurons, as reported previously in the medial vestibular nucleus (Kodama et al., 2012). The expression of transmitter-related genes and ion channel genes, including those controlling post-inhibitory rebound firing, are qualitatively and quantitatively differentiated across sampled MCN neurons, classifying them into at least three excitatory and three inhibitory cell types. Expression of marker gene candidates, such as *Spp1*, differed across cell types. Using such cell-type specific marker genes, we are identifying the projection targets of each cell type via retrograde tracing from known MCN targets, including parafascicular thalamic nucleus, periaqueductal grey, and lateral hypothalamus. Defining molecular and connectivity distinctions among medial cerebellar neurons will enable cell-type specific manipulation of motor, homeostatic, autonomic, and cognitive functions of the cerebellum.

Disclosures: H. Fujita: None. **T. Kodama:** None. **S. Guerrero:** None. **S. du Lac:** None.

Poster

561. **Cerebellum: Anatomy and In Vitro Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 561.26/CCC3

Topic: D.14. Cerebellum

Support: National Research Foundation of Korea Grants 2009-0080939

National Research Foundation of Korea Grants 2011-0030737

Title: Lobule-Specific differences of hyperpolarization-activated current and rebound depolarization in cerebellar Purkinje cell

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Abstract: Purkinje cells (PCs), which are known as core neurons in cerebellar motor learning, integrate and encode multimodal afferent inputs of both excitatory and inhibitory information. As each lobule in the cerebellar vermis receives different sensory inputs, the information would be encoded in a lobule specific way. We have already reported that the membrane excitability of PCs are significantly different between spinocerebellum (lobule III-V) and vestibulocerebellum (lobule X) . Although sensory inputs into PCs are mainly excitatory, PCs also receive massive inhibitory synaptic inputs from the local inhibitory interneurons. However, the influences of inhibitory inputs on PCs have not investigated systematically. In this study we compared the change of membrane properties in PCs in response to hyperpolarizing current injection between lobule III-V and lobule X. We performed electrophysiological recordings using patch clamp technique from fresh brain slices of cerebellar vermis. Recordings under voltage-clamp mode showed that hyperpolarization-activated current (Ih current) was significantly larger in lobule X than lobule III-V. Relative sag amplitude, which means the proportion of sag amplitude to maximum hyperpolarizing amplitude during negative current injection, was also significantly higher in lobule X than lobule III-V . We measured first spike latency of rebound depolarization (RD)-associated bursts, which was significantly shorter in lobule X than lobule III-V . The rebound slope from the end of hyperpolarizing current injection to the threshold of the first spike of RD-associated burst was -0.197, -0.230, -0.254 at -60mV, -65mV, and -70mV in lobule III-V, and -0.336, -0.331, -0.332 at -60mV, -65mV, and -70mV in lobule X, which was not significantly different . RD amplitude under 1 uM TTX was significantly different between lobule III-V and X. These results indicate that electrical properties of PCs in response to hyperpolarizing current injection are significantly different between lobule III-V and X, which implies that signal encoding of inhibitory inputs in PCs may be implemented lobule-specifically.

Disclosures: J. Shin: None. **C. Kim:** None. **J. Kim:** None. **S. Kim:** None.**Poster**

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.01/CCC4

Topic: D.16. Posture and Gait

Support: JSPS KAKENHI Grant-in-Aid for Scientific Research (B) (23300238)

Title: Postural control during transient floor translation while standing with the leg and trunk fixed

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Abstract: When subjects are exposed to a postural disturbance, it is important to anticipate the disturbance timing and to prepare for the postural control. Thus, we have investigated event-related potentials (ERPs) that contain the contingent negative variation (CNV) and postural muscle activation pattern during the floor translation task (Fujiwara et al. 2011; 2012). CNV peak time showed a high correlation with the start time of continuously increasing electromyogram (EMG) in the postural muscles just before S2. However, individual differences were recognized in the postural muscles, due to the postural control strategy (Fujiwara et al. 2011). If postural movement is restricted mainly to the ankle, having common postural strategy among individuals, then a higher correlation would be found between CNV peak time and activation timing of a specific postural muscle. In this study, a cast brace that fixes the joints in the leg and trunk, but not in the ankle was made for each subject, and the relationship between CNV and postural muscle activity during postural disturbance was investigated. Twelve healthy young adults repeatedly underwent forward postural disturbance by a backward floor translation (S2) 2 s after an auditory warning signal (S1). Speed and amplitude of floor translation were set, based on the fluctuation of the center of foot pressure in the anteroposterior direction (CoPap) during extreme forward leaning. Under the fixation and non-fixation conditions, a set of 10 translations was repeated, at least 4 sets, until CoPap mean position during floor translation in the final set did not differ from that in the former-set. CNV, CoPap and EMG of the postural muscles in the last two sets were analyzed. In both conditions, CNV peaked just before S2. Around the CNV peak, activity of the triceps surae and CoPap started to increase and move forward, respectively. After S2, CoPap rapidly displaced forward and then moved backward. With the fixation, the CNV peak amplitude increased and the forward displacement of CoPap after S2 significantly decreased. Onset time of the triceps surae after S2 became earlier with fixation. This may be due to an increase in background activity of this muscle just before S2. In addition, activation onset time of the triceps surae just before S2 showed a high correlation with CNV peak time. These changes in postural control may be related to focusing of attention on the ankle joint movement. These findings suggest that it would be useful to execute the backward transient floor translation with the trunk and leg fixed as a muscle training method to improve dynamic balance focused on the triceps surae.

Disclosures: N. Kiyota: None. **K. Fujiwara:** None. **M. Maekawa:** None. **M. Irei:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.02/CCC5

Topic: D.16. Posture and Gait

Title: Multi-muscle control of postural muscles: Common neural inputs

Authors: *A. SANTOS[1], C. LEONARD[1], A. DEGANI[2], V. CARDOSO[3], A. MAGALHAES[3]; [1]Sch. of Physical Therapy and Rehabil. Sci., The Univ. of Montana, Missoula, MT; [2]CNSAP, Londrina, Brazil; [3]Federal Univ. of Piaui, Parnaiba, Brazil

Abstract: The notion of muscle synergies has been viewed as a solution to the notorious problem of motor redundancy since it decreases the number of degree-of-freedom to be controlled by the postural system. Recently, it has been proposed that correlated neural input may be the mechanism used by the CNS to coordinate the formation of a synergistic group. In this particular study we investigated this hypothesis by analyzing the strength and distribution of correlated neural inputs to postural muscles previously recognized as synergistic for the execution of whole body tasks. Nine subjects performed the task of standing while holding a 5Kg barbell in front of their bodies at chest level. Subjects were asked to keep the quiet stand position as stable as possible for 10 seconds while the activity of four postural muscles were recorded by surface electrodes: Soleus (SOL), Gastrocnemius Medialis (GM), Biceps Femoris (BF), and Lumbar Erector Spinae (ERE). EMG-EMG pooled coherence estimates were computed taking into account five muscle pairs (SOL/BF, SOL/ERE, GM/BF, GM/ERE, and BF/ERE). The experimental conditions induced a significant increase in the muscle activation levels for all four muscles recorded. EMG-EMG coherence analysis revealed significant coherence estimates within three distinct frequency bands; <1Hz, 5-17Hz, and 23-45Hz. Significant coherence estimates significantly larger for lower frequency bands. These findings suggest the existence of common neural inputs distributed among multiple postural muscles forming a functional group. In addition, the occurrence of significant coherent muscle activation on multiple frequency bands suggests the existence of multiple sources involved on the generation of such common inputs.

Disclosures: A. Santos: None. **C. Leonard:** None. **A. Magalhaes:** None. **V. Cardoso:** None. **A. Degani:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.03/CCC6

Topic: D.16. Posture and Gait

Support: NSF BCS-0925878

AFOSR FA9550-12-1-0395

Title: Statistical analysis of quiet stance sway in 2-D

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Abstract: Spontaneous postural oscillatory behavior during quiet stance shows a stochastic, confined random-walk like trajectory. Its two-point temporal correlations, characterized by the stabilogram diffusion function (SDF), show a power law in the short time spans (τ) indicating positively correlated activity, and saturation at longer τ suggesting anti-correlation. Chow and Collins' (1995) 1-D stochastic continuum pinned polymer model (PPM) shows that such sway can be effectively mimicked by the dynamics of points along a flexible polymer with uniform density, under tension, driven by a stochastic forcing, while embedded in an elastic sheet with viscous drag that pins it to a local stable position. Our overall goal was to extend the 1-D PPM to a 2-D scenario, and use it and the SDF to assess adaptive learning.
Five healthy, adult subjects (age 20-65) passively stood in normal stance, eyes closed, on a force platform at the center of a rotating room. We compared the sway before (baseline), during, and after exposure to 10 rpm rotation. The parameters characterizing the SDF revealed adaptive changes in sway. At $\tau < 1$ s, the initial rise of the SDF showed power law dependence, $\tau^{1+0.5}$ to $\tau^{1+0.9}$, for AP and ML sway and all rotation periods. The absolute difference of the exponents was never more than 0.4. Onset and offset of rotation significantly increased both the intercept and the exponent compared to baseline. At $\tau > 1$ s, the SDF saturated to plateaus for all conditions except immediately after rotation onset where the SDF showed large variations. The SDF plateaus both during and after rotation were significantly different from baseline. A positive plateau residual of the post-rotation SDF versus baseline in the AP direction always correlated with a negative residual in ML, and vice versa, for all 5 subjects. The probability of this reciprocal ML/AP plateau pattern occurring by chance is 0.031. We extended Chow and Collins' 1-D PPM to an equivalent 2-D PPM because in a rotational environment the orthogonal Coriolis forces generated by sway movements lead to a 2-D sway coupling. Our 2-D PPM model correctly predicted the power scaling for the SDF and residuals for $\tau < 1$ for all the conditions. In future work, we will explore the relationship between the model parameters and adaptive learning and expand the 2-D PPM to predict the saturation levels at $\tau > 1$ s and explain the reciprocal relationship of the post-rotation AP and ML residuals.

Disclosures: A. Bakshi: None. **P. DiZio:** None. **J.R. Lackner:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.04/CCC7

Topic: D.16. Posture and Gait

Title: Muscle activation patterns in very slow walking are different from those of a natural gait

Authors: *R. MURAKAMI[1,2], T. KURAYAMA[1,2], Y. GOTO[1,2], Y. TANI[1,2], Y. TADOKORO[1], C. KONDO[1], K. SASAYA[1], D. MATSUZAWA[2], E. SHIMIZU[2], J. NISHII[3], K. KONDO[1], Y. OTAKA[1,4];
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Abstract: [Objectives] Whereas there are infinite combinations of step length and step rate when one walks at a given speed, adult humans usually walk at an invariant ratio of step length divided by step rate (walk ratio) over a wide range of speeds, with little inter-subject variability. It has also been found that the muscle activation patterns in gait are invariant with respect to gait speeds. Researchers have shown that only 4 or 5 basic components of muscle activation patterns account for the majority of muscle activities during gait. We have confirmed, however, that at very slow speeds, the walk ratios were larger than those at preferred and faster speeds and that there was more inter-subject variability. Those results imply that very slow gait might be regulated by a different mechanism. The aim of this study was to explore whether the components of the muscle activation patterns in very slow gait are the same as those in normal gait. [Methods] Thirteen healthy subjects were enrolled in this study. Participants were asked to walk on a treadmill at speeds of 10, 20, 40, 60, 80 and 100 m/min. We recorded muscle activities from 16 unilateral leg and trunk muscles using surface electrodes. The EMG signals of each muscle were then normalized in time with the gait cycle and averaged over 20 step cycles. In accordance with the procedure of Ivanenko et al (2004), we applied a principal component analysis (PCA) to the normalized EMG data sets of each gait speed. The step rates were obtained from a motion capture system and step lengths were calculated from those step rates with the given treadmill speeds. [Results] As reported previously, the results of the PCA indicated that four principle factors accounted for about 90% of the total EMG signals across 16 different muscles at the gait speeds of 60 m/min and 80 m/min. On the other hand, at slower or faster speeds, some component waveforms by the PCA were different from those of 60 m/min and 80 m/min. At the very low speeds, we could no longer derive the basic components. Statistically

significant differences in walk ratios between the very slow speed and the others were also observed (p<0.05). [Conclusion] The basic components of the muscle activation patterns in gait are not invariant at very slow speeds. Walking at very slow speeds may not be regulated by the same mechanism as the cyclically patterned movements of natural gait at normal speeds.

Disclosures: R. Murakami: None. **T. Kurayama:** None. **Y. Goto:** None. **Y. Tani:** None. **Y. Tadokoro:** None. **C. Kondo:** None. **K. Sasaya:** None. **D. Matsuzawa:** None. **E. Shimizu:** None. **J. Nishii:** None. **K. Kondo:** None. **Y. Otaka:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.05/CCC8

Topic: D.16. Posture and Gait

Title: A comparison of movement characteristics between kneeling gait and regular gait after hemiplegic stroke

Authors: *Y. GOTO[1,2], T. KURAYAMA[1,2], R. MURAKAMI[1,2], Y. TADOKORO[1], Y. TANI[1,2], K. SASAYA[1], C. KONDO[1], K. AIMOTO[3], D. MATSUZAWA[2], E. SHIMIZU[2], K. KONDO[2], Y. OTAKA[1,4];
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Abstract: [Background] Trainings in the kneeling position have been anecdotally used in physical therapy to improve postural control of patients with stroke. However, clinical evidence is lacking and the characteristics of movements in the kneeling position have not been well explored. The purpose of this study was to clarify the movement characteristics of gait in a kneeling position (kneeling gait) compared to those of regular gait in post-stroke patients. [Methods] Sixteen post-stroke patients (aged 65.0 ± 11.5 years) who could perform kneeling gait with or without assistance were enrolled in this study. The study was approved by the local ethics committee and all participants gave written informed consent. Participants were required to perform kneeling gait and regular gait at a self-selected comfortable speed. Surface electromyograms (EMGs) of the 6 gait-related proximal muscles from both sides were recorded. Center of mass (COM) displacements were measured using an optical marker at the spinous process of the third lumbar vertebra. The root mean square (RMS) of the EMG signals of 20 gait cycles in each muscle was obtained. Using the Wilcoxon signed-rank test, the RMS value in each

muscle and COM displacements were compared between kneeling gait and regular gait. The RMS ratio of the non-paretic side to the paretic side in each muscle was also compared between the two tasks. [Results] The COM displacement in the lateral direction was significantly larger in the kneeling gait compared to regular gait. On the other hand, the COM displacement in the vertical direction was significantly smaller in the kneeling gait compared to regular gait. There was no statistically significant difference in RMS values for any muscle between kneeling and regular gait. The RMS ratios of the non-paretic side to the paretic side showed a tendency for elevation in the kneeling gait compared to the regular gait. This tendency was statistically significant in the rectus abdominis and the gluteus medius. [Conclusion] Overall, no statistically significant differences in the muscle activations were found between the kneeling gait and regular gait at self-selected comfortable speeds in post-stroke patients. The results, however, indicate that there are some differences in utilization of the gait-related muscles between the kneeling gait and regular gait; namely, there is a tendency to rely on the non-paretic muscles more than the paretic muscles. These findings give us some information about the utility of the kneeling gait to enhance the gait-related muscle activations, though further investigation is necessary to apply the results to the whole post-stroke population.

Disclosures: Y. Goto: None. **T. Kurayama:** None. **R. Murakami:** None. **Y. Tadokoro:** None. **Y. Tani:** None. **K. Sasaya:** None. **C. Kondo:** None. **K. Aimoto:** None. **D. Matsuzawa:** None. **E. Shimizu:** None. **K. Kondo:** None. **Y. Otaka:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.06/CCC9

Topic: D.16. Posture and Gait

Support: NIH Grant HD048741

NIH Grant HD007414

Title: How dysfunctional is gait asymmetry? Association between metabolic cost and asymmetries in post-stroke walking

Authors: *J. M. FINLEY, A. J. BASTIAN;
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Abstract: Stroke survivors often have a slow asymmetric walking pattern. They also walk with an increased energetic cost relative to healthy, age-matched controls. It is often assumed that

spatiotemporal asymmetries are in some sense sub-optimal and therefore contribute to the increased energetic cost of walking in these individuals. As a result, one approach to locomotor rehabilitation is to alleviate asymmetries in foot-placement and timing. Although there is evidence that asymmetric walking patterns are energetically costly for healthy individuals, it remains to be seen if this holds true for stroke survivors. Here, we asked whether kinematic parameters describing post-stroke gait asymmetry explain the metabolic cost of walking beyond what is explained simply by the slowed speed. We used expired gas analysis to compute the metabolic power required to walk at four different speeds for 13 stroke survivors. Each participant walked at their self-selected speed, 80% and 100% of their self-selected speed, and their fastest comfortable speed. We also computed metabolic power for 10 age- and gender-matched control subjects who walked at the same speeds as their matched stroke survivor. Kinematic data from six, bilateral anatomical landmarks were recorded using a motion capture system and these data were used to compute the magnitude of the following kinematic variables characterizing interlimb asymmetry: step length difference, double support difference, difference in single limb support, and difference in swing times. A multiple regression was performed to determine the association between the dependent variable, metabolic power, and the independent variables of walking speed, group (stroke vs. control), and each of the kinematic parameters. The results of the regression revealed that speed, group, and step length difference were the only parameters that were associated with metabolic power. Speed alone predicted 73% and 82% of the variability in metabolic power in stroke survivors and controls respectively, and across all speeds, stroke survivors had a higher rate of energy use than controls. Step length difference was positively correlated with metabolic power and explained an additional 5% of the variability in power for stroke survivors. These findings highlight the influence of step length asymmetry on the metabolic cost of walking post-stroke, and provide a functional rationale for reducing asymmetry using interventions such as split-belt treadmill training.

Disclosures: J.M. Finley: None. **A.J. Bastian:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.07/CCC10

Topic: D.16. Posture and Gait

Support: NIH Grant HD048741

Title: A marching-walking hybrid induces adaptation of step symmetry on a treadmill

Authors: *A. LONG[1], J. FINLEY[2], A. BASTIAN[2];
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Abstract: Physical rehabilitation with a split-belt treadmill has the potential to correct for asymmetric stepping in stroke survivors. In split-belt adaptation, subjects walk on a treadmill with two belts, one of which is moving faster than the other. Healthy controls and cerebral stroke survivors can adapt their walking pattern during split-belt walking and de-adapt during post-adaptation exposure to regular treadmill walking. It is during post-adaptation that we see improvement in the symmetry of stepping in stroke survivors, though this effect is relatively short lived. Yet repetitive long-term training with the split-belt treadmill can improve post-stroke step length symmetry for months after training, suggesting that this might be a viable rehabilitation treatment (Reisman et al. 2013).
Split-belt treadmills are not easily accessible for physical therapy, whereas single belt treadmills are common. Here we asked if a level of adaptation similar to split-belt walking can be achieved by healthy subjects who walk with one foot on a moving belt while the other marches in place on a stationary belt (i.e. marching-walking hybrid). Subjects were required to march on the stationary belt to maintain a similar reciprocal gait cycle to that of normal walking. Subjects in the marching group adapted to a 1 m/s speed difference for 15 minutes and then de-adapted by walking with both belts at 0.5 m/s. A split-belt control group adapted to belt speeds of 0.5 and 1.5 m/s and then de-adapted with both belts moving at 0.5 m/s. This control group was exposed to the same difference in belt speeds as the marching group. Kinematics were recorded for leg and trunk movement using an Optotrak system. Step symmetry was calculated as the difference in step lengths measured from ankle to ankle.
Results showed that the major difference between the groups was the time course of adaptation and de-adaptation_the marching group was markedly slower compared with the walking group. Both groups experienced similar magnitude perturbations during early adaptation, achieved similar levels of step symmetry by the end of adaptation and showed similar magnitude aftereffects early in post-adaptation. These results indicate that a marching-walking hybrid can induce motor adaptation that is similar to that achieved on a split-belt treadmill. The fact that it takes longer to adapt and de-adapt may be advantageous for training stroke survivors, because it prolongs symmetric walking during post-adaptation in a single session. Furthermore, our findings suggest that physical rehabilitation with this march-walk paradigm potentially could be conducted on single belt treadmills. Supported by NIH HD048741.

Disclosures: A. Long: None. **J. Finley:** None. **A. Bastian:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.08/CCC11

Topic: D.16. Posture and Gait

Support: RO1AG029510

Title: Lateral stability for single and multiple step recovery responses to lateral perturbations of standing balance in older adults

Authors: *M. FUJIMOTO, W.-N. BAIR, M. PRETTYMAN, B. BEAMER, M. ROGERS; Physical Therapy & Rehabil. Sci., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: In prospective studies of older adults, an inability to recover from a lateral loss of balance with a single step is predictive of future falls. When balance is disturbed, the central nervous system appears to estimate current and future state of motion of the whole-body center of mass (COM) in relation to the base of support (BOS), which may determine the number and type of steps used to prevent falling. We hypothesized that the state conditions for dynamic stability at the instant of first step-off (SO) would differentiate multiple from single step recovery responses to lateral perturbations of standing balance.

Fifty-four healthy older adults received 60 randomly applied lateral waist-pull perturbations, while kinematic and ground reaction force data were recorded. Responses to the right lateral pull at 30cm/s were analyzed, where the largest number of subjects (n = 17) responded with crossover stepping using the left leg with both single and multiple steps. The COM and center of pressure (COP) positions in the medio-lateral direction were calculated with respect to the right foot BOS width. A single-link-plus-foot inverted pendulum model was used to define lateral stability boundary at SO. The stability boundary also was adjusted using the COP position at SO, considering it as a functional limit of the BOS. Stability margins were calculated as the shortest distance from the experimental data to the stability boundary defined based on both the actual and functional limits of the BOS. A paired t-test was performed to examine differences between single and multiple step responses with a significance level of 0.05.

At SO, a step was initiated later, relative to the pull onset, for multiple step responses than single steps ($p=.051$), resulting in a more laterally located COM ($p=.003$), that was closer to the lateral stability boundaries. Consequently, stability margins were significantly smaller for multiple steps than single steps ($p=.019$). Furthermore, the stability margin for multiple steps was not significantly different from zero when the functional limit was used for the stability boundary estimation, which implies that there was no margin for lateral stability at SO. This suggests that the stability margin based on the functional limit rather than the actual limit of the BOS better differentiated multiple from single step responses. These findings indicate that different protective stepping responses could be predicted by the COM motion state as early as first step-off. While stability margins could differentiate multiple from single step responses, the use of the functional limit provides a more sensitive estimation of the lateral stability boundary.

Disclosures: M. Fujimoto: None. **W. Bair:** None. **M. Prettyman:** None. **B. Beamer:** None. **M. Rogers:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.09/CCC12

Topic: D.16. Posture and Gait

Support: JSPS KAKENHI Grant Number 24700611

Title: Developmental changes in activation patterns of postural muscles during bilateral arm flexion

Authors: *T. KIYOTA[1], K. FUJIWARA[2], K. KUNITA[3], K. ANAN[3], C. YAGUCHI[4];
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Abstract: We investigated developmental changes in activation patterns of postural muscles during bilateral arm flexion. A total of 176 subjects participated in this study (number of subjects (n): 4 years: n = 27; 5 years: n = 35; 6 years: n = 42; 7-8 years: n = 23; 9-10 years: n = 17; 11-12 years: n = 32). Subjects stood on the force platform with closed stance. In response to a visual stimulus (LED signal) presented at 1-3 s after a warning signal, the subjects initiated bilateral arm flexion as quickly as possible and then stopped their arms voluntarily at a horizontal position. After 5 practice trials, 10 test trials were performed with a 30 s-rest between the trials. EMG activity of the following muscles were recorded from surface electrodes: the anterior deltoid (AD), rectus abdominis, erector spinae (ES), rectus femoris, biceps femoris (BF), tibialis anterior (TA), and soleus. The reaction time of the activation onset of AD (D0) in response to the LED signal was measured. Time difference between D0 and the activation onset in the postural muscles were calculated as the start time of postural muscles. Activation rate calculated as the percentage of trials with the burst activation among 10 trials. AD reaction time in 4 years was the longest in all age groups, and the reaction time was significantly shortened with age. A significant effect of age was observed for the start time of ES, BF and TA. The start time of ES and BF was shortened with age, while TA was lengthened. The start time of ES was significantly earlier than D0 after 6 years. The start time of BF was significantly later than D0 in every age group. The start time of TA showed no significant difference with D0 in 4 years, while after 5

years, it was significantly later than D0. The activation rate of ES and BF was more than 90% in every age group and that of TA was about 50%. In adults, the activation onset of ES and BF precedes that of the focal muscles (AD) during bilateral arm flexion (Fujiwara et al., 2003). However, in the children, the preceding activation was observed in ES only and there was no preceding activation of BF even in the latter half of the childhood. Additionally, in adults, the activation rate of TA was less than 20% (Fujiwara et al., 2003). On the other hand, even in 11-12 years, children present results showed the relatively high activation rate of TA (about 50%). These results suggest that developmental changes in the postural muscle activation during bilateral arm flexion in the childhood is earlier in the trunk than in the thigh and lower legs, and even in the latter half of the childhood, the postural control accompanied by the muscle activation in the thigh and lower legs has not attained the adult-like postural control yet.

Disclosures: T. Kiyota: None. **K. Fujiwara:** None. **K. Kunita:** None. **K. Anan:** None. **C. Yaguchi:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.10/DDD1

Topic: D.16. Posture and Gait

Title: Alterations in dynamic postural stability with exhaustive repetitive sit-to-stand exercise

Authors: *M. BRYANTON[1], M. BILODEAU[2];
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Abstract: Dynamic multi-articular activities of daily living, such as rising from a chair (sit-to-stand; STS), involve the displacement of limb segments and in turn one's centre of mass (COM). The STS movement not only requires the development of adequate resistive torques, but also precise control of one's vertical projection of COM over a rapidly reducing support surface at the moment of seat off. The purpose of this study was to investigate alterations in dynamic postural control due to fatigue accumulated from exhaustive repetitive STS movements. Voluntary dynamic range testing was performed in 10 healthy young adults, which involved measuring the extent to which each individual could voluntarily shift their centre of pressure (COP) towards the toes and heels without losing balance, under both eyes open (EO) and eye closed (EC) conditions. Maximal and minimal COP coordinates were collected before (PRE), immediately after (POST) and 10 min (RECOV) after a STS fatigue protocol. The fatigue protocol involved repetitive STS to a standardized pace until volitional exhaustion occurred. COP coordinates as

well as ankle, knee and hip joint angle positions were collected at moment of seat off throughout the fatigue protocol. Dynamic range testing indicated that although the EC condition significantly reduced dynamic range, and increased variance ($p=0.001$, and $p=0.002$ respectively) in comparison to the EO conditions, a significant fatigue effect was only seen with an increase in variance measures between PRE and POST ($p=0.004$). Because the STS movement requires higher efforts from the knee extensor in comparison the ankle plantar-flexor and hip extensor musculature, this finding suggests that dynamic voluntary range measurements are most likely dependant on ankle plantar-flexor force control capabilities, while accumulation of fatigue in the larger primary agonist muscle such as the quadriceps may have a role in reduced stability as indicated by increased variance when attempting to maintain the COP within the BOS. In turn, STS fatigue trial data indicated a significant anterior shift of the COP ($p=0.027$), which can be interpreted as a stabilization strategy occurring at seat off due to reduce knee extensor contractile performance with progression of fatigue. Based on previous investigations, it was expected that this would be associated with a compensatory reduction in knee flexion and an increased hip flexion, however, no significant changes in joint angles at moment of seat off were observed ($p>0.05$).

Disclosures: M. Bryanton: None. **M. Bilodeau:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.11/DDD2

Topic: D.16. Posture and Gait

Support: NIH R01-AR046386

NIH P30-GM103333

Title: Activation differences during internal and external rotational knee moments during standing target matching tasks in healthy and ACL injured patients

Authors: A. S. LANIER, K. MANAL, *T. S. BUCHANAN;
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Abstract: While many people have surgery to treat knee ligament ruptures (ACL deficiencies), others are able to cope with these injuries and do well without surgery because they adopt different neural control strategies. These individuals have been identified as *copers* and respond differently to tasks involving dynamic stabilization of the knee (Rudolph et al., 1998). The neural

control of knee joint stability involves controlling internal and external rotation (IER) of the tibia relative to the femur and improper control of IE stability has been associated with the development of osteoarthritis (Henrikksen et al., 2012). We are studying how ACL injured subjects compensate for internal and external rotations during a standing target matching task. In this study we compare IER knee moments of three groups: healthy uninjured controls, those with ACL deficiency 1 year after injury who have not had surgery (ACL-d 1yr), and those who have undergone reconstruction (ACL-r).
The 3 subject groups included 8 healthy subjects (4M, 4F), 5 ACL-d 1 yr. subjects (5F), and 8 ACL-r subjects (5M, 3F). During each test, subjects stood barefoot with each foot on a separate force plate. They were given visual feedback of the AP and ML shear forces that one foot exerted on the force plate, visualized by a cursor. Subjects were instructed to move the cursor representing these forces into a target that represented force in the AP-ML plane. Targets appeared at 20° increments around a circle with zero force at the center. Forces in the contralateral limb, the limb not controlling the cursor, were used in the data analysis, as this was the limb of interest. The cursor was required to be at each target for 500ms, and a total of 72 targets were examined. The IE knee moment was determined in the contralateral limb (the one *not* controlling the cursor) using inverse dynamics.
We observed that the shear forces from healthy and ACL-r knees were statistically indistinguishable. However, subjects who did not receive ACL reconstructions (ACL-d 1yr subjects) activated their muscles to have significantly greater IER knee moments compared to both healthy and ACL reconstructed knees, especially during forces involving anterior shear. This implies that subjects with ligament deficiencies use different strategies to stabilize their joints.

Disclosures: A.S. Lanier: None. **T.S. Buchanan:** None. **K. Manal:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.12/DDD3

Topic: D.16. Posture and Gait

Support: Ataxia UK

Ataxia Ireland

German Hereditary Ataxia Foundation (DHAG)

Oliver-Vaihinger-Fonds, Stiftung für kranke Kinder, Tübingen

Title: Whole-body controlled video games improve dynamic stability in children with degenerative cerebellar disease

Authors: *W. ILG[1], C. SCHATTON[1], B. MUELLER[1], N. LUDOLPH[1], L. SCHOELS[2], M. A. GIESE[1], M. SYNOFZIK[2];
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Abstract: Background: The cerebellum is crucial for motor control (e.g. of gait and posture) and motor learning. Therefore, motor rehabilitation in patients with degenerative cerebellar disease is challenging, and the capability of motor improvements for these patients is not fully understood. We have recently shown, that a 8 weeks motor training program based on playing whole-body controlled video games can lead to a reduction of ataxia symptoms and an improvement in gait in children with degenerative cerebellar disease (Ilg 2012). In this study, we examined quantitatively, whether this motor training leads to - specific improvements in motor control of complex whole-body movements, which are relevant in everyday life and which cannot be explained simply by improvements in general fitness
Methods: To assess the specific effects of motor training, we analyzed the movement behavior during playing the Xbox Kinect™ game "Light Race" of 10 children with degenerative cerebellar disease versus 10 age-matched controls. Here, subjects have to control an avatar performing one minute sequences of rapid stepping movements towards different goals. Cerebellar children were tested in this game before and after an 8 weeks training program including different video games focusing on dynamic balance, trunk-limb coordination and goal-directed movements. The rapid stepping sequences during game playing were analyzed with respect to dynamic stability (Hof 2005), multi-joint coordination, anticipatory postural adjustments and movement variability.
Results: After 8 weeks training, children improved their general game play with respect to games scores, increased averaged velocity and dynamic stability. In addition, specific measures revealed (a) improved anticipatory postural adjustments before stepping ($p=0.04$), (b) decreased movement decomposition ($p=0.01$), (c) decreased movement variability during stepping ($p=0.04$) as well as increased dynamic stability at the end of the stepping movements ($p=0.01$).
Conclusion: Despite progressive cerebellar degeneration children are able to improve specific aspects of motor performance in complex whole-body movements which are relevant in everyday life (e.g. rapid stepping movements to compensate for gait perturbations). Therefore, directed training of whole-body controlled video games present a highly motivational, cost-efficient and home-based rehabilitation strategy to train dynamic balance, multi-joint coordination and interaction with dynamic environments in a large variety of young-onset neurological conditions.
References:

Hof A, et al. J Biomech 38: 1-8, 2005.
Ilg W, et al. Neurology 79: 2056-2060, 2012.

Disclosures: W. Ilg: None. **C. Schatton:** None. **B. Mueller:** None. **N. Ludolph:** None. **L. Schoels:** None. **M.A. Giese:** None. **M. Synofzik:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.13/DDD4

Topic: D.16. Posture and Gait

Title: Assessment of Forces in the palm of the hand in amputee football players during crutch walking, running and shooting

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Abstract: Introduction: In amputee football players have one leg amputee/limb deficiency and use bilateral crutches. Instead of daily prosthesis, amputee football athletes depend on the Loftstrand crutches on the field during walking, running and shooting. The forces on the body are significantly increased at forearms and hands during ambulation with crutches. Since they utilize their arms for bearing body weights, players have to cope with the high loads encountered during high-speed crutch running or shooting. Thus, in these individuals strength of the upper extremity is a key factor to minimize shoulder, elbow and wrist injuries.
Aim: The purpose of the study was to measure forces at the palm of the hand in the stance phase during walking, running and shooting for seven professional amputee soccer players.
Method: The study included seven licensed amputee football players (amputation duration: 42.1±29.3 months; height 172.5±7.97 cm; weight: 63.0±12.6 kg; BMI 21.05±3.07) with unilateral left leg amputation/limb deficiency. The subjects were asked to walk and jog at a comfortable pace on a 20-meter track with Lofstrand crutches. Using an acceleration distance of 3 m, athletes performed penalty shots with crutches.
A F-Grip system (Tekscan Inc, boston, USA) equipped with high-resolution sensors built into a glove was used to assess the impact forces. Dynamic forces at the palm of the hand were recorded during walking, running and penalty shooting.

Results: The measured forces at the palm of the hand were significantly higher immediately before the penalty kick with crutches ($p<0.05$) compared to the running condition. There were no significant differences between the side of amputation and the healthy side regarding palm forces ($p>0.05$).
Conclusions: In the game, Lofstrand crutches transmit forces to the hand and can cause upper extremity injuries that might be correlated with body weight and increasing speed during the stance phase. In order to prevent injuries in amputee football players, the determination of high loads encountered during each movement pattern is important and training regimens should be designed and optimized for increasing strength of the upper extremity.
In addition a proper crutch gripping strategies should be taught to players that may enable them to squeeze the crutches forcefully only during the stance phase of the crutches to further prevent related injuries.

Disclosures: S. Uzun: None. **N. Ramazanoglu:** None. **Y. Tatar:** None. **F. Camliguney:** None. **C. Karagozoglu:** None. **A. Pourmoghaddam:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.14/DDD5

Topic: D.16. Posture and Gait

Support: CONACyT Grant 261504

Title: Differential effect of chronic undernutrition on the postnatal development of gait in male and female wistar rats: A kinematic study

Authors: V. MARTÍNEZ-ÁLVAREZ[1], J. GUADARRAMA[2], B. SEGURA-ALEGRÍA[3], *M. ALVARADO[1], I. JIMÉNEZ-ESTRADA[2];
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Abstract:
This study was aimed to analyze the possible effect of chronic undernutrition on the postnatal development of the gait in female and male rats. Adult Wistar were maintained under different feeding conditions: A) Control group: the rats and their offspring had free access to water and food. B) Undernourished group: three weeks before mating and during gestation, lactation and post-weaning periods the puppy rats were fed with half the food ingested by control animals (each group n=8 per litter). The kinematic analysis of gait activity was performed once a week,

for 9 weeks from the 4th or 5th day of life of the offspring (the day of birth was considered as P0). Once quantified the weight, height and body mass, the following hindlimb joints were inked with a permanent nontoxic: whilst, hip, knee, ankle, and toe, then the unrestricted animal gait was videotaped over an acrylic passeway. The Cartesian coordinates of each joint were determined frame by frame by using the ImageJ program (NIH). Using a video-assisted computerized system developed in our lab it was measured the length, duration and speed of each animal stride (data obtained from stride length and speed was normalized according to the body mass of animals). Chronic food deprivation induces a significant reduction in the morphometric parameters of male (P> 0.01) but less in female rats. At post-weaning stages of development, the gait of undernourished male rats is characterized by strides of lower duration (25-30%) but higher normalized length (146.7 to 199.0%) and notorious higher stride speed (158.0 to 657.1%) than the strides of control male rats (P> 0.01). Meanwhile, the gait of undernourished female rats was practically similar to that of control female rats, at all the postnatal ages analyzed.
According to our results, it is suggested the existence of gender differences in the alterations evoked by chronic undernutrition on the postnatal development of gait in the rat.

Disclosures: V. Martínez-Álvarez: None. **J. Guadarrama:** None. **B. Segura-Alegría:** None. **M. Alvarado:** None. **I. Jiménez-Estrada:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.15/DDD6

Topic: D.16. Posture and Gait

Support: NIH/NIAMS AR053608

Title: The neural control of joint state in the rat

Authors: *M. C. TRESCH[1], B. RELLINGER[2], T. SANDERCOCK[3];
[1]Biomed Engin. & Physical Med. & Rehab, Northwestern Univ., CHICAGO, IL; [2]Biomed. Engin., [3]Physiol., Northwestern Univ., Chicago, IL

Abstract: Understanding why the central nervous system (CNS) uses a particular set of muscle activations during behavior is usually considered in the context of how muscles affect task level variables: how does activation of a muscle contribute to the limb kinematics or joint torques

necessary to achieve desired task goals? However, muscles also affect variables related to joint state, such as contact forces between bones or ligament strains. We examine here whether such joint state variables might also be considered by the CNS when determining muscle activation patterns.

We cut the nerve to vastus lateralis (VL) in the rat and examined the adaptations in EMGs and kinematics over a period of 2 months. EMGs were implanted in up to 15 hindlimb muscles and recorded during treadmill locomotion. In separate experiments, we have shown that because of the deep patellar groove in the rat VL and vastus medialis (VM) produce very similar joint torques across the skeleton when stimulated in vivo. Rectus femoris (RF) has a similar action at the knee as these muscles, but also produces a hip flexion torque. If the CNS regulated only joint torques, one would expect compensation to loss of VL to involve increased activation of VM, avoiding the hip torque produced by RF. However, we have also shown that VM and VL produce distinct mediolateral forces on the patella. Note that these different patella forces have no direct effect on task performance. If the CNS regulated joint state variables related to patellar forces, one would expect compensation to involve increased RF activation since RF does not produce a mediolateral force on the patella.

In 3 of 4 animals, we found that loss of VL initially (1-3 days) caused a substantial reduction in knee extension, along with substantial alterations in ankle and hip angle trajectories. Activation of RF and VM was increased while early stance phase activation of hamstring muscles was reduced. Following 2 months of adaptation, hip and ankle kinematics were similar to those before the cut and knee extension was mostly restored. Activation of RF remained increased but activation of VM returned to levels similar to those observed before the cut, as did activation of hamstring muscles. The preferential increase in RF at later time points suggests that the CNS does regulate joint state variables, minimizing unbalanced forces on the patella. However, activity in VM was not eliminated, suggesting either an incomplete adaption or that regulation of joint torques also played a role in determining adaptation patterns. These results suggest that joint state variables, in addition to task relevant variables, are used by the CNS to determine muscle activation patterns during behavior.

Disclosures: M.C. Tresch: None. **B. Rellinger:** None. **T. Sandercock:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.16/DDD7

Topic: D.16. Posture and Gait

Title: Altered cortical excitability during trunk postural control

Authors: *S. GOTTARDI, S.-Y. CHIOU, P. H. STRUTTON;
Imperial Col. London, London, United Kingdom

Abstract: The muscles of the trunk become active during limb movements, to stabilise the torso; this is known as postural adjustment. These muscles are also activated during voluntary movements of the trunk such as flexion and extension. The extent to which the primary motor cortex is involved in controlling these muscles in each of these different tasks is unclear. Thus, the purpose of the present study was to investigate the corticospinal excitability of the back muscles during tasks with differing postural components.
Twenty healthy adults were recruited (10 female; mean age: 21.7). Bilateral electromyographic (EMG) activity was recorded from erector spinae (ES) at vertebral level L4, rectus abdominis and anterior deltoid. Subjects performed 3 tasks separately; dynamic shoulder flexion (DSF), static shoulder flexion (SSF) and static trunk extension (STE). Transcranial magnetic stimulation was applied over the hot-spot for the contralateral ES muscles at 120% active motor threshold during the tasks. Ten motor evoked potentials (MEPs) were obtained during each task. The background EMG in the ES muscle of interest was measured during the DSF and this was matched by the subject for the static tasks using visual feedback. The procedure was repeated on the other hemisphere. Corticospinal excitability was assessed by measuring the amplitudes of the ES MEPs, these were compared for differences across tasks.
There were significant differences in the MEP amplitudes between the 3 tasks for both hemispheres ($P<0.001$). With right hemisphere stimulation, the MEP amplitudes in the left ES were significantly larger in the DSF (mean±SD mv:0.64±0.41) compared to the other tasks (0.36±0.35, SSF and 0.41±0.44, STE; $P<0.001$). Similarly, with left hemisphere stimulation, the MEP amplitudes in the right ES were significantly larger in the DSF (0.59±0.40) compared to the other tasks (0.36±0.27, SSF and 0.38±0.31 STE; $P<0.001$). The MEP amplitudes were not significantly different between the 2 static tasks for either hemisphere.
The results of the present study indicate that during the dynamic task there is a greater degree of motor cortical involvement controlling the trunk muscles than during the static tasks. This supports the growing body of evidence of cortical involvement in postural control. However, it is not clear why the static task with a postural component (SSF) did not show an increase in cortical excitability over that seen in the voluntary trunk contraction. Overall, these results may be pertinent to patients with low back pain, where altered postural control and cortical excitability have been reported.

Disclosures: S. Gottardi: None. **P.H. Strutton:** None. **S. Chiou:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.17/DDD8

Topic: D.16. Posture and Gait

Title: Head control is affected by trunk and neck bracing during gait

Authors: *S. MORRISON[1], K. KELLERAN[2], D. RUSSELL[3];
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Abstract: When walking, an ongoing control problem faced by the human neuromuscular system is to ensure the impact of gait-related oscillations on head motion is minimized. One possible means by which the system minimizes head motion during gait is through the damping properties of the trunk. The aim of this study was to investigate the effect constraining motion of the trunk and neck (through external bracing) has on 3D head acceleration during walking. Twelve healthy adult individuals were required to perform 3 straight-line walking trials along a 70m level walkway at their preferred walking speed. Changes in movement dynamics were assessed under four conditions; 1) Control (no bracing), 2) Neck brace only, 3) Trunk brace only, and 4) Both neck and trunk braced. Three lightweight tri-axial accelerometers, attached over the occipital pole (head), C7 spinous process (neck), and L3 spinous process (trunk) were used to measure trunk and head acceleration during the walking activity. Data analysis involved assessing changes in amplitude (mean RMS), regularity (Approximate Entropy, ApEn) and smoothness (harmonic ratio) of the respective acceleration signals as a function of the bracing condition. As predicted, the combined effect of bracing both the neck and trunk had the greatest impact on head acceleration patterns and general gait dynamics. Under these more restricted conditions, the pattern of regularity (ApEn) and smoothness (harmonic ratio) of the head acceleration signal in the vertical, ML and AP directions was notably different from that observed under the unbraced (control) conditions. Together these results illustrate that restricting the motion of trunk-neck segments has a significant impact on head motion, and that these changes have a top-down effect for the overall gait dynamics.

Disclosures: S. Morrison: None. **K. Kelleran:** None. **D. Russell:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.18/DDD9

Topic: D.16. Posture and Gait

Title: Investigating lower extremity functioning via frontal plane movement variability asymmetries during landing

Authors: *A. D. NORDIN, J. S. DUFEK;
Kinesiology and Nutr. Sci., Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: The purpose of this research was to examine bilateral differences in frontal plane lower extremity range of motion (ROM) variability during drop landings. Movement variability was used to assess neuromotor functioning, the ability of the motor system to vary internal loads, and subsequent injury susceptibility. Group and single-subject analyses were carried out to explore motor responses to varied task demands, explored through changes in landing height. Kinematic movement variability was assessed via frontal plane lower extremity joint ROM at the hip, knee and ankle joints. Fourteen participants (7 male, 7 female; mean age 22.64±3.41 years; height 1.71±0.10m; mass 67.56±10.31kg) free from previous lower extremity injury were examined. Participants completed 5 bilateral drop landing trials at 5 successive drop heights, computed as a percentage of maximum vertical jump height (MVJH; 20%, 60%, 100%, 140%, 180%). Kinematic data were acquired using a 12-camera Vicon system (200Hz), and 35 marker spatial model. Variability was expressed using coefficient of variation. Comparisons were made via a group 2x3x5 (limb x joint x height) repeated measures ANOVA, as well as 2x5 (limb x height) repeated measures ANOVAs for each participant. Group analysis revealed a significant interaction between limb and height ($F(2,26)=18.36$, $p<.001$, $\eta^2=.586$), where the left (32.8±17.1%) and right limbs (23.7±13.2%) differed at the 20%MVJH landing height ($t(41)=2.95$, $p=0.005$). Additionally, a significant main effect for lower extremity joint demonstrated that the knee (26.1±15.8%, $p=.009$) and ankle joints (20.0±12.6%, $p<.001$) showed significantly lower frontal plane ROM variability relative to the hip (35.4±17.2%). Single-subject analyses highlighted a significant limb by height interaction in a single participant ($F(4,8)=3.98$, $p=.046$, $\eta^2=.666$). Similarly, a significant main effect for limb was shown in a single participant ($F(1,2)=20.91$, $p=.045$, $\eta^2=.913$), while significant main effects for height were shown among 2 participants ($F(4,8)=9.20$, $p=.004$, $\eta^2=.821$, $F(4,8)=6.34$, $p=.013$, $\eta^2=.760$, respectively). From this examination, it is suggested that although the grouped model provides insight into the average movement variability response to alterations in task demands, single-subject analyses allow insight into motor functioning at the level of the individual, potentially demonstrating susceptibility to injury as a result of inter-limb control differences. This avenue of research has practical implications, but also sheds light into fundamental control processes associated with gross motor tasks, which shows considerable variation among individuals.

Disclosures: A.D. Nordin: None. **J.S. Dufek:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.19/DDD10

Topic: D.16. Posture and Gait

Title: Control of landing during forward-induced stepping for balance recovery in healthy young adults

Authors: *M. INACIO, R. CREATH, M. ROGERS;
Physical Therapy and Rehabil. Sci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Protective stepping is a commonly used strategy to stabilize posture and prevent falls when balance is disturbed. Previous results suggest that elderly subjects who respond to balance perturbations with multiple steps are more likely to fall. This study investigated the dynamic features of the first-step landing for single and multiple balance recovery steps after forward waist-pull perturbations for young healthy adults in order to determine the importance of first-step landing in successfully preventing multiple recovery steps.
Twelve healthy young adults received forward waist-pulls via a computer-controlled robotic puller at a magnitude chosen to induce a stepping response. Recovery responses were classified as successful (single step) or unsuccessful (multiple steps) at foot contact (FC), peak vertical ground reaction force (FzPeak), and the interval between these events (BTWN). Kinematic and kinetic data were used to determine body segment and center of mass (COM) motion characteristics, as well as ankle, knee, and hip joint moments and powers. Between-group comparisons were performed using independent samples t-tests (significance: $p \leq 0.05$).
Six subjects were successful and six unsuccessful in preventing multiple recovery steps. Both groups demonstrated similar body segment kinematics and first step length. The unsuccessful group tended to use a wider step. Peak net joint moments were higher for the unsuccessful group at BTWN and FzPeak for the stepping-limb ankle, knee and hip joints. Except for BTWN, the unsuccessful group produced more positive net power for ankle, knee and hip joints while the successful group appeared to prevent multiple steps by absorbing energy at the hip joint. For successful stepping trials subjects had a more posteriorly-extended trunk orientation at FzPeak. Although vertical COM motion and momentum were not significantly different between the groups, antero-posterior COM velocity and momentum tended to be higher in the unsuccessful group. For the unsuccessful stepping trials subjects had a more anteriorly-positioned COM and smaller stability margins, which tended to progressively increase from FC to FzPeak.
The results of this study suggest that: 1) the ability to successfully prevent multiple recovery steps and arrest forward body motion following forward balance perturbations is dependent on first-step landing dynamics; 2) even among healthy younger adults there are individuals who rely on multiple steps to recover their balance. Future efforts will apply these results to elderly high

fall-risk individuals to determine if task-specific training can improve first-step landing dynamics and reduce falls.

Disclosures: M. Inacio: None. **R. Creath:** None. **M. Rogers:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.20/DDD11

Topic: D.16. Posture and Gait

Support: Parkinson's Disease Foundation

Doris Duke Foundation

Title: Kinematic strategies underlying step responses to postural perturbations

Authors: R. A. MCGOVERN[1], J. CORTES-RAMIREZ[2], P. GREENE[2], G. M. MCKHANN II[1], *P. MAZZONI[2];
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Abstract: Taking a step is a major strategy used by humans to recover balance when posture is perturbed. While this response involves a complex set of well-characterized biomechanical, muscular, and kinematic components, a quantitative description at the level of controlled motor variables is lacking. We hypothesized that the stepping response is functionally a kinematic task, and specifically: 1) the disturbance that is ultimately relevant is the initial movement of the body's center of mass (COM), and not the magnitude of disturbing force; 2) the response that mediates recovery is appropriate placement of the stepping foot relative to the moving COM, regardless of forces exerted by the feet; 3) scaling relationships should exist among specific kinematic variables that characterize disturbance and response. Therefore we sought to provide a kinematic account of postural stepping by applying a postural perturbation and studying the initial body motion and the subsequent movements of the stepping feet.
We tested 7 young healthy subjects on a modified version of the clinical pull test and recorded their movements with a motion capture system. Subjects were pulled from behind multiple times, each time with an intensity randomly selected across a wide range. We then tested for correlations among kinematic variables for the COM's initial motion and for the step response. All subjects recovered balance in one step. The stepping foot's placement was tightly linked to the COM's position and velocity at the time of foot landing, consistent with a predominantly kinematic strategy for posture recovery. Of the variables that determine foot placement, step size

was strongly correlated with perturbation intensity, and this correlation was greatest with peak initial COM acceleration. Correlations with COM acceleration were weaker for reaction time (foot lift-off time), and absent for step duration. The postural step response may thus be described as taking a step whose amplitude is scaled to the kinematics of the postural disturbance in order to stop the body's motion. This scaling relationship offers a functional description of the normal postural step response, and may be a candidate descriptor of response adequacy in patients with specific types of postural instability.

Disclosures: R.A. McGovern: None. **P. Mazzoni:** None. **J. Cortes-Ramirez:** None. **P. Greene:** None. **G.M. McKhann II:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.21/DDD12

Topic: D.16. Posture and Gait

Title: Postural coordination pattern as a function of scaling the surface of support dynamics

Authors: *J. KO, J. H. CHALLIS, K. M. NEWELL;
The Pennsylvania State Univ., University Park, PA

Abstract: This study investigated the organizational properties of postural control system as a function of the continuously scaled support surface dynamics. We examined how the number and nature of the dynamical degrees of freedom in the movement coordination patterns changed as a function of the frequency of the support surface and a practice. 12 young adults stood on a moving platform that was sinusoidally translated in the anterior-posterior (AP) direction. Dynamics of the support surface was driven by its frequency that was continuously scaled up from 0.2 Hz to 1.3Hz within a trial at a fixed 16cm amplitude. Task was to maintain postural balance on the moving platform having practice for 3 days. 4 joint angular motions (ankle, knee, hip, and neck) were recorded by 3D motion analysis system and used to run principal component analysis (PCA) to estimate the number of dynamical degrees of freedom of the postural coordination patterns. Continuous relative phase was quantified to investigate coordination patterns between two joint angular motions. PCA revealed that, in early practice, the modal number of dynamical degrees of freedom of the coordination pattern was 3 in the lower frequency conditions (< 0.7 Hz) and 2 or 3 in the higher frequency conditions. With practice, however, the coordination patterns were organized by 2 dynamical degrees of freedom in the higher frequency conditions (> 0.8 Hz). The relative phase analysis revealed that the ankle-hip

coordination was highly variable in the lower platform frequency (0.2 ~ 0.5Hz), whereas it showed the anti-phase pattern () with low variability as a function of the platform frequency and practice. However, the ankle-knee and knee and hip coordination did not show any particular patterns having higher variability across the platform frequencies and practice. Continuously scaled support surface dynamics induced different organizational properties of coordination patterns and it was influenced by practice. Particularly, in the higher frequency conditions, postural control system was constrained by the stable ankle-hip coordination to preserve postural stability. This study has revealed how the postural control system manages a number of joint space degrees of freedom for redundant postural tasks and provided evidence on a particular optimal solution to preserve postural stability.

Disclosures: J. Ko: None. **J.H. Challis:** None. **K.M. Newell:** None.

Poster

562. **Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, Biomechanics**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 562.22/EEE1

Topic: D.16. Posture and Gait

Support: Grant-in-Aid for Young Scientists (B) (#22700568) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan

Grant-in-Aid for Young Scientists (B) (#25870164) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan

Title: Inter-joint dynamic interaction during human quiet standing examined by induced acceleration analysis

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Abstract: Recent studies have demonstrated that controlling human quiet standing is one of the multi-joint movements (Hsu et al. 2007; Pinter et al. 2008; Sasagawa et al. 2009), in which the central nervous system (CNS) is required to deal with dynamic interaction among the joints to achieve desired motor performance. The purpose of this study was to investigate how the CNS deals with such inter-joint interaction during quiet standing by examining causal relationship between kinetics (torques) and kinematics (angular accelerations) within the multi-degree-of-freedom body. In this study, the standing body was approximated as a double-link inverted

pendulum (with ankle and hip joints) rotating in the sagittal plane. We conducted a novel analysis called “induced-acceleration analysis” (Zajac et al. 2002) to quantify the mutual contributions of net torques (sum of the joint torque, angular-velocity dependent torque, and gravitational torque) of the ankle and hip joints to induce the angular accelerations in each joint. As a result, we found that the net ankle and hip torques induced angular accelerations of comparable magnitudes in each joint. In addition, the angular accelerations induced by the net ankle and hip torques were consistently counter-directed to one another in each joint. With such quantitative and temporal relationships, the angular accelerations induced by the two net torques counter-compensated, and thus, the amplitudes of resultant, measured angular accelerations of the individual joints were substantially reduced. These results suggest that the CNS may take advantage of inter-joint interaction to reduce combined effects of the individual net torques on each joint motion during quiet standing.

Disclosures: S. Sasagawa: None. **M. Shinya:** None. **K. Nakazawa:** None.**Poster**

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.01/EEE2

Topic: D.17. Voluntary Movements

Support: ORF RE04-47

Title: Perturbation motor corrections correlate with features of reaching and proprioception impairments post-stroke

Authors: *T. BOURKE[1], S. D. BAGG[2], S. P. DUKELOW[5], K. E. NORMAN[3], S. H. SCOTT[4]; [1]Ctr. for Neurosci. Studies, Queens Univ., Kingston, ON, Canada; [2]Physical Med. and Rehabil., [3]Sch. of Rehabil. Therapy, [4]Dept. Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada; [5]Univ. of Calgary, Calgary, AB, Canada

Abstract:
Limb afferent feedback is used for perception of our limb geometry and for motor control. Stroke can damage sensory and motor regions of the brain leading to impairments of upper limb function. Our previous work found that deficits in position sense were independent of deficits in reaching performance. This independence is surprising given that limb afferent feedback plays an important role in voluntary motor action. The objective of the present study was to explore in more detail the relationship between sensory and motor impairments in subjects with stroke using three distinct behavioural tasks.
Subjects with subacute stroke (n=18) and non-disabled controls (n=79) were assessed in a

KINARM exoskeleton robot that permits motion in the horizontal plane. A postural perturbation task measured how quickly
subjects can respond to a perturbation and accurately return to their start
target (no vision of hand following perturbation). A limb matching task
assessed the perception of limb position by measuring the mismatch between one arm moved by the robot and the subject's mirror matched limb configuration with their other arm (no vision throughout task). A visual-guided reaching task assessed the use of vision and proprioception to reach quickly and accurately to a target. Both arms were assessed in each task and a number of parameters captured the spatial and/or temporal aspects of task behavior.
In agreement with our previous results, reaching and position matching deficits were found to be largely independent in this sample. Most impairments in reaching parameters were significantly correlated with impairments in perturbation corrections. In contrast, the majority of impairments in limb matching parameters were significantly independent of impairments in perturbation corrective parameters. One exception was that perturbation response endpoint error was significantly correlated with limb matching variability ($r=0.43$, $p<8.8x10^{-4}$).
Overall these results suggest impairments in integrating feedback into motor corrections being related to motor impairments and independent of proprioceptive impairments.

Disclosures: T. Bourke: None. **S.P. Dukelow:** None. **K.E. Norman:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies. **S.D. Bagg:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.02/EEE3

Topic: D.17. Voluntary Movements

Title: Using clinical and robotic assessment tools to examine the feasibility of pairing tDCS with standard physical therapy in patients with stroke and TBI

Authors: J. A. MIDDLETON, D. LIUZZO, R. NEWMAN-NORLUND, S. L. FRITZ, *T. M. HERTER;
Dept. of Exercise Sci., Univ. of South Carolina, Columbia, SC

Abstract: Introduction: Following neurologic insult, such as a stroke or traumatic brain injury (TBI), an imbalance in excitation and inhibition between the affected and unaffected cortical hemispheres may contribute to functional impairments. Bihemispheric transcranial direct current stimulation (tDCS) may diminish this imbalance by exciting cortical neurons on the affected side of the brain and inhibiting cortical neurons on the unaffected side. Purpose: A case series design was used to examine the feasibility of pairing bihemispheric tDCS with standard upper extremity physical therapy in a heterogeneous sample of individuals with chronic stroke and TBI. Methods: Five individuals with chronic stroke, one individual with TBI, and one individual with stroke resulting from a TBI participated in 24 sessions of standard UE physical therapy (40 minutes, 3 times per week). Bihemispheric tDCS at 1.5mA was delivered over motor cortex during the first 15 minutes of therapy. Outcomes were assessed using clinical (UE Fugl-Meyer, Purdue Peg Board, Box and Block, Stroke Impact Scale) and robotic (position sense, unimanual and bimanual motor control, and executive function) assessments at 8 time points (2 pre-intervention, 2 interim, and 1 post-intervention, and 3 follow-up). Results: Two participants with chronic stroke did not complete the study due to unrelated illness. Of the remaining participants with stroke, one exhibited significant improvements in robotic measures of unimanual and bimanual motor control whereas two did not show significant recovery. The participant with TBI demonstrated improved bimanual coordination and the participant with stroke resulting from TBI demonstrated improvements on clinical and robotic measures of unimanual motor control. All three participants who exhibited significant recovery maintained their improvements at follow-up. Conclusions: Our results show that bihemispheric tDCS may be a feasible, effective adjunct to standard physical therapy for providing sustainable improvement in upper extremity function following stoke and TBI. This intervention may not be effective for individuals with moderate to severe spasticity. Robotic assessment may improve our ability to monitor longitudinal recovery in individuals who respond to treatment.

Disclosures: J.A. Middleton: None. **S.L. Fritz:** None. **T.M. Herter:** None. **D. Liuzzo:** None. **R. Newman-Norlund:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.03/EEE4

Topic: D.17. Voluntary Movements

Support: Heart and Stroke Investigatorship in Stroke Rehabilitation Research (to SPD)

CIHR MOP - 106662

CIHR NSP - 104015

Heart and Stroke Foundation of Alberta, Northwest Territories, Nunavut GIA

Title: Comparison of post-stroke position sense and kinaesthesia using robotics

Authors: *J. A. SEMRAU[1], T. M. HERTER[2], S. H. SCOTT[3], S. P. DUKELOW[1];
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Abstract: Proprioception allows us to use both static position sense and sense of dynamic limb motion (kinaesthesia) to properly plan and execute movement. Prior work in our lab has shown that in subjects with stroke, damage to both of these aspects of proprioception is common, occurring in ~50% of stroke survivors. While proprioception has classically been sub-divided into position sense and kinaesthesia, it is likely that their integration is key to perceiving sense of limb location and movement. Here, we aim to further characterize the relationship between these two submodalities of proprioception to better understand their contributions to the control of movement and how they are impacted by stroke.

One-hundred neurologically intact control subjects and 110 subjects with unilateral stroke performed two separate robotic tasks. In both tasks, subjects sat in the KINARM robotic exoskeleton with their arms supported against gravity, vision occluded. Task 1: Position Matching - The robot moved one arm to one of nine locations in a virtual workspace. Subjects were instructed to use their opposite arm to mirror-match the position that the robot moved their passive arm to, after the robot stopped moving. Task 2: Kinaesthetic Matching - The robot moved one arm to one of three locations in the virtual workspace at a preset speed, direction and magnitude of movement. Subjects were instructed to mirror-match with their opposite arm the speed, direction, and magnitude of movement as soon as they felt the robot begin to move their arm. For all subjects with stroke, the robot moved their affected arm.

We evaluated spatial parameters of static position sense and static kinaesthesia, as well as dynamic (spatial and temporal) components of kinaesthesia. Preliminary results suggest a strong relationship between deficits in position sense and kinaesthesia, with 45% of stroke subjects impaired on both the kinaesthesia task and position matching. This result is further supported by significant correlations of position matching task measures of variability with kinaesthestic parameters of directionality and response latency ($p < 0.001$). Additionally, we observed significant correlations in position matching variability with kinaesthetic measures of endpoint variability ($p < 0.001$). These results suggest that both static and dynamic components of proprioception play a pivotal role in proper sensory function. Additionally, these components are likely highly integrated due to the high incidence of coincidental impairment of both position sense and kinaesthesia. By using robotics to identify sensory deficits we hope to better inform post-stroke rehabilitation and treatment strategies.

Disclosures: J.A. Semrau: None. **T.M. Herter:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder and creator of KINARM. **S.P. Dukelow:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.04/EEE5

Topic: D.17. Voluntary Movements

Support: Heart and Stroke Investigatorship in Stroke Rehabilitation Research (to SPD)

CIHR MOP - 106662

CIHR NSP - 104015

Heart and Stroke Foundation of Alberta, Northwest Territories, Nunavut GIA

Title: The neural correlates of position sense after stroke

Authors: *S. FINDLATER[1], J. A. DESAI[1], J. A. SEMRAU[1], T. M. HERTER[2], S. H. SCOTT[3], S. P. DUKELOW[1];
[1]Clin. Neurosciences, Univ. of Calgary, Calgary, AB, Canada; [2]Univ. of South Carolina, Columbia, SC; [3]Queen's Univ., Kingston, ON, Canada

Abstract: ***Background:*** Proprioception describes our sense of self-position and movement without the use of vision. It is impaired in approximately one-half of stroke patients. Proprioceptive deficits have been correlated with poor motor recovery after stroke and have been linked to impaired function resulting in longer inpatient stays and increased care requirements upon discharge. Diagnosis and quantification of the severity of proprioceptive deficits poses a challenge in the clinical setting, given that the most widely used bedside assessments have been found to have poor sensitivity, reliability, and lack normative data. We have developed and validated an objective robotic assessment of position sense, a sub-component of proprioception, in subjects with stroke.
Recently, voxel-based lesion-symptom mapping (VLSM) has been used to examine the correlation between brain lesion location and behavioural deficits on a voxel-by-voxel basis. This study used VLSM to examine the relationship between lesion location and impaired position sense after stroke.
Methods: 70 subjects completed the robotic assessment within 3 weeks of their stroke. Position

sense was measured using a matching task where the KINARM robotic exoskeleton moved the affected arm to one of nine locations. Subjects were instructed to mirror match the position with their unaffected arm without the use of vision. The results of the position match task were compared with lesion location on magnetic resonance images, acquired within 10 days of stroke, using VLSM.
Results: As hypothesized, damage to the internal capsule, somatosensory cortex, and somatosensory association cortex (areas 5 and 7) were associated with poor position sense. Further, lesions within the insula, external capsule, and middle frontal gyrus were also observed in association with impaired position sense.
Conclusion: Our results are consistent with traditional views of proprioceptive anatomy while suggesting the involvement of additional structures. Identification of brain regions involved in processing position sense may prove useful to clinicians and researchers who are interested in understanding the role that sensory information plays in stroke recovery.

Disclosures: S. Findlater: None. **J.A. Desai:** None. **J.A. Semrau:** None. **T.M. Herter:** None. **S.P. Dukelow:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); founder and creator of the KINARM.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.05/EEE6

Topic: D.17. Voluntary Movements

Support: Heart and Stroke Investigatorship in Stroke Rehabilitation Research (to SPD)

CIHR MOP - 106662

CIHR NSP - 104015

Heart and Stroke Foundation of Alberta, Northwest Territories, Nunavut GIA

AHFMR ITG 200801019

Title: Identification of brain areas associated with impaired kinaesthesia following stroke using robotics and voxel-based lesion symptom mapping

Authors: *J. M. KENZIE[1], J. A. SEMRAU[1], S. E. FINDLATER[1], J. A. DESAI[1], T. M. HERTER[2], S. H. SCOTT[3], S. P. DUKELOW[1];

[1]The Univ. of Calgary, Calgary, AB, Canada; [2]Univ. of South Carolina, Columbia, SC; [3]Queen's Univ., Kingston, ON, Canada

Abstract: To interact with our environment, we require proprioception - the sense of limb position and movement (kinaesthesia). Clinically, it is difficult to measure kinaesthesia in an objective and sensitive way. Previous work in our lab has developed robotic methodology to measure kinaesthesia and we have found that ~50% of stroke survivors have impairments. Here, we utilize both robotics and imaging-based analysis (voxel-based lesion symptom mapping (VLSM)) in stroke survivors to identify brain areas associated with processing kinaesthetic information.

Seventy-eight subjects with stroke performed a kinaesthetic matching task using the KINARM robotic exoskeleton. MRI/CT imaging and robotic scores were obtained for stroke subjects within 10 days and 21 days post-stroke, respectively. For the kinaesthesia task, the robot moved the subjects' stroke-affected arm at a preset speed, direction and magnitude of movement. Subjects then mirror-matched the movement with their unaffected arm as soon as they felt the robot begin to move. VLSM was used to statistically compare the location of the brain lesions with behavioural scores from the robot task.

We observed significant associations between impaired kinaesthesia and lesions of internal and external capsule, insular cortex, and Broadmann's areas 5 and 7. Several of these structures have previously been found to be important for transmission and processing of kinaesthetic information. Our findings suggest that a widely distributed network is responsible for processing and integrating spatial and temporal elements for sense of limb movement. By identifying brain structures associated with poor kinaesthesia following stroke, we may better predict which individuals with acute stroke will have kinaesthetic deficits.

Disclosures: J.M. Kenzie: None. **J.A. Semrau:** None. **S.E. Findlater:** None. **J.A. Desai:** None. **T.M. Herter:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder and creator of KINARM. **S.P. Dukelow:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.06/EEE7

Topic: D.17. Voluntary Movements

Support: CIHR NSERC Collaborative Health Research Program

Title: Influence of elbow angular spasticity zones on one-trial motor learning in chronic stroke

Authors: *S. K. SUBRAMANIAN[1,2], A. G. FELDMAN[3,2], M. F. LEVIN[1,2];
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Abstract: Motor learning studies in subjects with stroke have mainly focused on the less-impaired arm to separate confounding influences of motor deficits from motor learning capabilities. One previous study assessed motor learning (error correction) in the hemiparetic arm using a one trial learning paradigm ─ rapid adaptation of arm motion to a new load. It was shown that error correction strategies were related to both cognitive and arm motor impairment. Subjects with mild paresis, like healthy subjects corrected movement errors elicited by the new load after one trial, while those with moderate to severe paresis used different strategies or were unable to completely correct movement errors. We hypothesized that resistance of muscles due to spasticity may be responsible for altered correction strategies. Zones in shoulder-elbow angular space in which muscles have spasticity revealed by passive muscle stretches and abnormal agonist/antagonist activation during voluntary movements have been identified by determining the threshold joint angles at which stretch reflex (SR) responses occurred despite the instruction to relax muscles. We assessed the influence of spasticity zones on motor learning in stroke. Stroke subjects (Fugl-Meyer scores: 32-61/66) made rapid 35-50° horizontal elbow extension movements from an initial 3° to a final 6° target in 16 blocks. Each block consisted of 6-10 trials each. In Session 1, movements were made in mid-range and in Session 2, movements were restricted to a joint range that did not surpass the flexor SRT (outside of the spasticity zone). For each block, movements were alternatively not loaded or loaded by a position dependent load (30% MVC). Subjects were instructed to extend the elbow to the final target in a single fast and accurate movement and correct movement errors as soon as possible. Visual feedback of elbow position and movement speed was provided. Angular positions before correction were used to identify error correction strategies. Changes in load condition from load to no load and vice-versa resulted in overshoot and undershoot errors respectively. In Session 1, subjects corrected errors in 1-4 trials. When movements occurred outside of the spasticity zone, the number of trials needed to correct errors fell to 1-3 with the majority needing only 1-2 trials. Results of this study may help distinguish arm motor learning ability from errors due to the underlying motor deficits in the paretic arm. The presence of spasticity zones should be taken into account during interpretations of motor learning results.

Disclosures: S.K. Subramanian: None. **A.G. Feldman:** None. **M.F. Levin:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.07/EEE8

Topic: D.17. Voluntary Movements

Support: CIHR Grant

Title: Altered obstacle avoidance behaviour in individuals with good arm recovery after stroke

Authors: *M. C. BANINA[1,2], B. J. MCFADYEN[3,4], M. F. LEVIN[1,2];
[1]Sch. of Physical and Occup. Therapy, McGill Univ., Montreal, QC, Canada; [2]Feil-Oberfeld Res. Centre, Jewish Rehabil. Hosp., Ctr. for Interdisciplinary Res. in Rehabil. of Greater Montreal, Montreal, QC, Canada; [3]Dept. de réadaptation, Faculté de médecine, Univ. Laval, Québec, QC, Canada; [4]Ctr. Interdisciplinaire de Recherche en Réadaptation et Intégration Sociale, Québec, QC, Canada

Abstract: After stroke, individuals with good sensorimotor recovery of their affected arm report decreased use of the arm in activities of daily living. Decreased use of the affected arm may be associated with undetected motor deficits only identifiable when individuals attempt higher-order tasks that require complex coordination. One higher-order motor task, the ability to avoid obstacles while reaching, commonly occurs in everyday environments but is not routinely assessed by clinical scales. We hypothesized that well-recovered people after stroke would be less successful in avoiding an obstacle in the reaching path compared to age-equivalent healthy controls. Obstacle avoidance ability during reaching in a virtual environment (VE) was compared between well-recovered stroke subjects and healthy controls. A VE was developed simulating a grocery store aisle and a commercial refrigerator with sliding doors stocked with bottles on 2 shelves. Subjects reached as fast as possible with their affected/dominant arm for a bottle on one shelf (non-obstructed reaching - template). In random trials (RAND, 30% of 60 trials), the door ipsilateral to the reaching arm closed and partially obstructed the bottle at reach initiation. Subjects were instructed to touch and retrieve the bottle without the hand or arm hitting the door. Arm and trunk movements were recorded with 24 active markers by an Optotrak system. Outcome variables were overall success rates, movement performance and movement quality variables for template (T), successful avoidance (Succ), and failed avoidance (Fail) trials, and Succ/Fail divergence points of the endpoint trajectory from template profile (DP=% of reach distance). In T trials, stroke subjects used less wrist flexion, wrist abduction and shoulder rotation compared to controls. In RAND, 36% of controls and 12% of stroke subjects were successful >65% of the time ($z=2.248$; $p<0.05$). For both groups, successful door avoidance was characterized by DP occurring closer to the starting position (Control: DPSucc=11.2±7.0%, DPFail=34.1±37.3%, $p<0.05$; Stroke: DPSucc=20.5±16.1%, DPFail=60.4±33.7%, $p<0.05$). However, the margin of error in the stroke group was about half that of the controls. In addition,

stroke subjects had to significantly increase endpoint trajectory length compared to controls to successfully avoid the door. Stroke subjects had residual movement deficits that were revealed through a challenging motor task. The potential of using challenging UL tasks to identify higher order motor control deficits should be considered when assessing post-stroke motor recovery.

Disclosures: M.C. Banina: None. **M.F. Levin:** None. **B.J. McFadyen:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.08/EEE9

Topic: D.17. Voluntary Movements

Support: Canadian Physiotherapy Foundation

Collaborative Health Research Projects

Univalor

Title: Responsiveness of a new clinical measure of spasticity

Authors: A. A. MULLICK[1,2], A. K. BLANCHETTE[2,3], R. GUBEREK[2], C. BEAUDOIN[2], *P. S. ARCHAMBAULT[1,2], M. F. LEVIN[1,2];
[1]Sch. of Phys & Occ Therapy, McGill Univ., Montreal, QC, Canada; [2]Feil and Oberfeld Res. Center, Jewish Rehabil. Hospital, Ctr. for Interdisciplinary Res. in Rehabil. (CRIR), Montreal, QC, Canada; [3]Dept. of Rehabil., Univ. Laval, Quebec city, QC, Canada

Abstract: While spasticity affects over 12 million people worldwide, there is still no universally accepted tool for its quantification. Spasticity is defined as a velocity-dependent increase in tonic stretch reflexes with exaggerated tendon jerks, resulting from hyper-excitability of the stretch reflex as one component of the upper motor neuron syndrome. However, existing spasticity measures do not adequately quantify these reflex properties. Levin and Feldman (1994) proposed a new approach to spasticity measurement based on the evaluation of the threshold position of body segments at which motoneurons begin to be recruited. This spatial threshold is the tonic stretch reflex threshold (TSRT) and is regulated by descending and segmental influences in a task-specific way, a concept underlying the equilibrium-point theory. Limitations in the regulation of TSRTs lead to motor deficits. In particular, spasticity occurs when the TSRT lies within the biomechanical range of the joint and cannot be regulated outside this range to relax muscles. Active muscle resistance emerges when muscles are stretched beyond the SRT. The

validity and reliability of the TSRT has been measured in the elbow and ankle in patients with post-stroke spasticity. To further develop the TSRT measure for clinical use, we estimated the responsiveness of the measure to changes in spasticity after a clinical intervention (TENS─ transcutaneous electrical stimulation). TENS is thought to decrease spasticity by increasing presynaptic inhibition of motoneurons over repeated applications. Patients with chronic post-stroke spasticity (≤75yr) were matched on elbow flexor spasticity level (Composite Spasticity Index-CSI) and randomly allocated to one of two groups; TENS/sham-TENS in a Pre-Post controlled design. TENS (high frequency-80Hz; low intensity-2x sensory threshold, 60 min) or sham-TENS (no stimulus delivery, but lights activated) was applied to spastic muscle antagonists (triceps). Subjects were instructed to use a portable TENS machine at home on a daily basis for 2 weeks. After 1 and 2 weeks of TENS/sham-TENS, changes in spasticity were measured by TSRT and clinical scales (Fugl-Meyer score, FM; Chedoke McMaster; CSI; Reaching Performance Scale). Between-group differences were analyzed by repeated measures ANOVA. The relationship between changes in TSRT and clinical scores was determined. Our results contribute evidence towards establishing the psychometric properties of TSRT as an objective clinical spasticity measure.

Disclosures: A.A. Mullick: None. **M.F. Levin:** None. **P.S. Archambault:** None. **C. Beaudoin:** None. **R. Guberek:** None. **A.K. Blanchette:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.09/EEE10

Topic: D.17. Voluntary Movements

Support: Collaborative Health Research Projects

Canadian Physiotherapy Foundation

Univalor

Title: Validity and reliability of the tonic stretch reflex threshold as a measure of ankle plantarflexor spasticity after stroke

Authors: *A. K. BLANCHETTE[1,2], K. MOÏN-DARBARI[2,3], A. A. MULLICK[2,4], C. BEAUDOIN[2], M. F. LEVIN[2,3,4];
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Hospital, Ctr. for Interdisciplinary Res. in Rehabil. (CRIR), Montreal, QC, Canada; [3]Neurosci., [4]Sch. of Physical and Occup. Therapy, McGill Univ., Montreal, QC, Canada

Abstract: One of the common impairments observed after neurological lesions is spasticity. Spasticity is defined as "a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex" (Lance 1980). Using an appropriate clinical measure for spasticity is important to evaluate the effectiveness of therapeutic interventions. The validity and reliability of commonly used clinical scales (e.g. Ashworth Scales [AS]) are questionable. Determination of the spatial tonic stretch reflex threshold (TSRT) is a promising objective alternative for spasticity measurement. The TSRT corresponds to the joint angle at which the stretch reflex response begins. In healthy subjects, it is broadly regulated by descending and spinal pathways, as specified in the equilibrium-point theory of motor control. Limitations in the range of regulation of TSRT in post-stroke subjects result in motor disorders, such as abnormal muscle activation patterns and spasticity. The objectives of the study were to: 1) quantify inter-rater reliability of the TSRT measure in ankle plantarflexors (PF) and 2) determine the correlation between spasticity measured with TSRT and clinical scales (AS, Composite Spasticity Index [CSI]). Thirty individuals (9 females; mean age: 57.1±9.8 yrs) with post-stroke ankle spasticity participated. PF spasticity was evaluated by two raters. Each rater manually stretched ankle PFs at different velocities (n=20, randomized). PF EMG activity and ankle angular position were recorded with surface electrodes and an electrogoniometer, respectively. For each stretch, the velocity-dependent dynamic stretch reflex threshold (DSRT), representing the joint angle at which muscle recruitment begins, was computed. TSRT was estimated by extrapolating a regression line through all DSRTs to zero velocity. Inter-rater reliability was good for ankle PF spasticity (two-way random ICC=0.80, $p<0.001$). There was no correlation between TSRT values and AS scores while a high correlation was found with the CSI score ($r=-0.506$; $p<0.005$). Determination of TSRT values is a valid and reliable method that can be a good alternative for the evaluation of spasticity since it is a physiological measure that accounts for the velocity-dependence of the stretch reflex response.

Disclosures: A.K. Blanchette: None. **K. Moïn-Darbari:** None. **C. Beaudoin:** None. **M.F. Levin:** None. **A.A. Mullick:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.10/EEE11

Topic: D.17. Voluntary Movements

Support: NFNZ Grant 0931-PG

Title: Priming physiotherapy with Theta Burst Stimulation enhances upper limb function in chronic stroke patients

Authors: *S. J. ACKERLEY[1,2], W. D. BYBLOW[3,2], P. A. BARBER[1,2], C. M. STINEAR[1,2]; [1]Dept. of Med., [2]Ctr. for Brain Res., [3]Dept. of Sport and Exercise Sci., Univ. of Auckland, Auckland, New Zealand

Abstract: Upper limb impairment is common after stroke and recovery of function is important for regaining independence in daily activities. This study tested the effects of priming upper limb therapy with intermittent Theta Burst Stimulation (iTBS), which is a form of non-invasive brain stimulation. We enrolled 18 patients with first-ever chronic monohemispheric stroke, in this randomized, controlled, single-centre, double-blinded trial. Priming consisted of real or sham iTBS delivered to the ipsilesional M1 immediately before 45 minutes of individualized upper limb physiotherapy. Primed therapy sessions were delivered on 10 consecutive working days. Upper limb impairment and function were assessed twice at baseline, after 5 (MID) and 10 (POST) primed therapy sessions, and at 4 and 12 weeks after the intervention. Upper limb impairment was assessed with the Fugl-Meyer scale (FM), and function was assessed with the Action Research Arm Test (ARAT). The primary endpoint was change in ARAT score 4 weeks after the intervention. Motor evoked potential amplitudes obtained in either first dorsal interosseous (FDI) and precision grip function were also assessed. Improvement in ARAT score was greater in the primed than control group patients at all time points after baseline ($F_{1,16} = 11.64$, $P = 0.004$), including the primary endpoint ($t_{16} = 2.29$, $P = 0.036$). Secondary analyses found that the primed group had a larger increase in ARAT score than the control group after 10 therapy sessions ($t_{16} = 3.78$, $P = 0.002$) but not after 5 therapy sessions ($t_{16} = 1.53$, $P = 0.15$). ARAT scores returned to baseline 3 months after the intervention. FM scores improved after 10 therapy sessions for both groups ($F_{3,48} = 3.41$, $P = 0.025$), and remained higher than baseline 3 months after the intervention ($t_{17} = 2.34$, $P = 0.032$). Ten sessions of upper limb therapy primed with iTBS boosts upper limb function in patients at the chronic stage of stroke recovery. The benefits last for at least 1 month, but primed therapy may need to be repeated periodically in order to produce a sustained benefit.

Disclosures: S.J. Ackerley: None. **W.D. Byblow:** None. **P.A. Barber:** None. **C.M. Stinear:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.11/EEE12

Topic: D.17. Voluntary Movements

Support: HRC Grant 09/164R

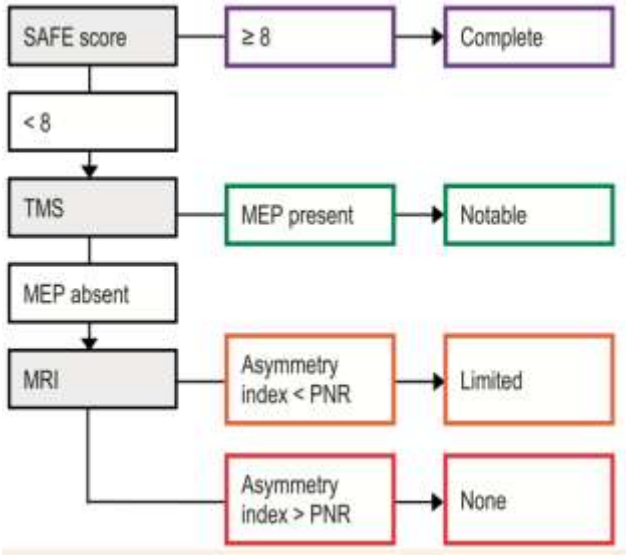
Stroke Foundation

Title: Does tractography improve prediction of individual patient's motor recovery after stroke?

Authors: *J. P. COXON[1], C. M. STINEAR[2], M. A. PETOE[2], P. A. BARBER[2], S. S. ANWAR[3], W. D. BYBLOW[1];
[1]Ctr. for Brain Res. & Dept. of Sport & Exercise Sci., [2]Ctr. for Brain Res. & Dept. of Med., Univ. of Auckland, Auckland, New Zealand; [3]Rehab Plus, ADHB, Auckland, New Zealand

Abstract: Predicting whether an individual will regain function of the upper limb after stroke is difficult. The PREP algorithm (for Predicting Recovery Potential, Figure) can be used within the initial days after stroke to predict functional outcomes for the individual patient at 3 months. PREP involves the sequential use of a bedside clinical assessment, transcranial magnetic stimulation of the lesioned hemisphere motor cortex, and diffusion-weighted (DW-) MRI to obtain fractional anisotropy (FA) of the posterior limbs of the internal capsules. The positive predictive power of this approach is good. Our aim was to determine if tractography of corticospinal (CST) and cortico-rubral pathways (CRP) in either hemisphere could be quantified in a way that would further improve PREP's positive predictive power. We examined 57 patients after first ever monohemispheric ischemic stroke. Clinical assessments were made within 72 hours, motor evoked potentials (MEPs) from TMS obtained at 5 days, and DW-MRI obtained within 10 days. All assessments were repeated at 12 weeks. In a small subset of cases, outcomes at 12 weeks exceeded or fell short of predictions. These cases tended to be where MEPs in the affected wrist extensor were present but FA asymmetry was high, or where MEPs did not modulate with stimulation intensity, had prolonged latencies, or both. In most cases tractography of the CST and CRP was unable to resolve uncertainty related to the presence or absence of MEPs. These results indicate that functional measures of CST integrity obtained from affected side MEPs are more salient than FA asymmetry obtained using DW-MRI. The results do not warrant the addition of tractography for predicting recovery potential in individual patients. The PREP algorithm rarely overestimates an individual's prognosis. Interestingly, DW-MRI revealed markers of structural plasticity within the contralesional CST for the group but this was not associated with improved paretic upper limb function, and may instead reflect increased use of

the nonparetic side after stroke.



Disclosures: J.P. Coxon: None. **C.M. Stinear:** None. **M.A. Petoe:** None. **P.A. Barber:** None. **S.S. Anwar:** None. **W.D. Byblow:** None.

Poster

563. Voluntary Motor Control: Stroke

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.12/EEE13

Topic: D.17. Voluntary Movements

Support: Project "Rehab_Mechanisms" funded by the Fondazione Pisa

Title: A semi-automated tool to study limb kinematics of reaching in a mouse model of stroke

Authors: *S. MICERA[1,2], S. LAI[2], A. PANARESE[2], C. SPALLETTI[3], C. ALIA[3], A. GHIONZOLI[2], M. MAINARDI[3], M. CALEO[3];
[1]Ecole Polytechnique Federale De Lausanne, Lausanne, Switzerland; [2]Scuola Superiore Sant'Anna, Pisa, Italy; [3]CNR Neurosci. Inst., Pisa, Italy

Abstract: Mice represent an ideal model to investigate the mechanisms of physiological and functional changes occurring after stroke. Currently, assessment of post-stroke motor impairments and recovery in mice is done by administering a series of behavioural tests, which results are usually evaluated by visual assessment of successful/unsuccessful movements, or by qualitatively studying limb kinematics by means of time consuming frame-by-frame video

analyses.
In this work, we propose a semi-automated algorithm for tracking mouse paw movement during a skilled reaching task, without attaching markers to the skin. The performance of this method was tested both before and after the induction of a focal ischemic stroke. Animals were trained to reach for food pellets with their preferred paw, previously painted with a non-toxic dye. During experimental sessions (lasting 5 min), mice were able to perform an average number of 30 reaching movements. A high frame rate (120 fps) camera was used to record the task. Later, a semi-automated algorithm written in Matlab was used off-line to automatically identify paw position and to track whole reaching trajectories. Inputs by the experimenter were limited to 1) an initial calibration during which the user was required to specify paw position in a few (e.g., 3 or 4) randomly selected frames, and 2) a quick review of successful/unsuccessful trials based on salient task points automatically identified by the algorithm. Various significant parameters were then extracted from the trajectories to assess motor performance similarly to what is currently done with patients after stroke (Panarese et al., Neurorehabil Neural Repair 2012).
Phototrombosis was then utilized to induce a focal ischemic stroke in the Caudal Forelimb Area, which affected forelimb motor performance. Mice were tested again once a week for 30 days. Significant differences were found between pre- and post-stroke values in a selected number of parameters.
This study presented and highlighted the utility of a semi-automated algorithm to study limb kinematics of mice reaching movements unhindered by markers in a quick and unbiased way. Furthermore, key parameters sensitive to post-stroke modifications in forelimb movements were identified. Our results suggest promise for using this method in stroke rehabilitation protocols for mice (e.g., Spalletti and Lai et al., Neurorehab Neural Repair, in press), in particular to assess whether functional gains occur via motor recovery (i.e., restoration of kinematic parameters to pre-injury values) or compensatory strategies.

Disclosures: S. Micera: None. **S. Lai:** None. **C. Spalletti:** None. **A. Panarese:** None. **C. Alia:** None. **A. Ghionzoli:** None. **M. Mainardi:** None. **M. Caleo:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.13/EEE14

Topic: D.17. Voluntary Movements

Support: Research Funding Development Grant, WVU

Title: Quantifying post-stroke movement impairment using motion capture to automate the Wolf Motor Function Test

Authors: *E. V. OLESH[1], S. YAKOVENKO[2], V. GRITSENKO[2];
[2]Ctr. for Neurosci., [1]West Virginia Univ., Morgantown, WV

Abstract: According to the National Stroke Association there are currently 7 million people living in the U.S. who have survived a stroke, half of whom suffer from long term movement impairment. With the increasing cost of rehabilitation and the lack of objective outcome measures of therapy effectiveness, there is a critical need for a low-cost and automated assessment that can provide accurate measurement of motor impairment. We have tested the feasibility of objectively scoring the upper extremity performance using low-cost motion capture. Stroke survivors performed 15 arm movements that are part of the Wolf Motor Function Test (WMFT). Each of these movements were scored by a group of physical therapy (PT) students on a 6-point scale (0 equalling no movement and 5 equalling perfect movement). The total score of 75 indicated no impairment. Subjects performed the WMFT with both arms and were recorded simultaneously by a video camera and a marker less motion capture system (Microsoft Kinect). Motion capture data consisted of 3D coordinates of 10 tracked points on the upper-extremities and the trunk. Joint angles of the shoulder, elbow and wrist were calculated with custom algorithms in Matlab (MathWorks). The analysis objective was 1) to compare the motion capture-based scores to those of PT students and 2) to determine the effectiveness of the WMFT in measuring the full range of motion. To objectively score the Functional Ability domain of WMFT, joint angle changes for shoulder flexion/extension, shoulder abduction/adduction, elbow flexion/extension and wrist flexion/extension were compared between the affected and unaffected limbs using Root Mean Squared Error (RMSE). The cumulative RMSE values for all movements determined a total score (0 indicates no impairment). The comparison of clinical scores to the motion capture-based scores showed a strong negative correlation. We used the span of joint angle from the unaffected arm to determine the difference between the range of motion captured by the WMFT and the physiological range of motion. Results from this multidimensional comparison demonstrate that the WMFT covers a significant portion of the full range of motion of the arm, and that portion diminishes with motor deficits. This study demonstrates the feasibility of using a low cost motion capture system to automate the WMFT and to objectively quantify post-stroke motor impairment.

Disclosures: E.V. Olesh: None. **S. Yakovenko:** None. **V. Gritsenko:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.14/EEE15

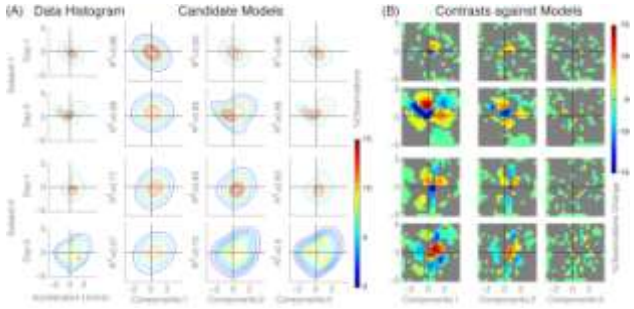
Topic: D.17. Voluntary Movements

Support: NINDS 2R01NS035673

Title: Distribution analysis reveals individual patterns of motor deficits

Authors: J. L. PATTON[1], *F. C. HUANG[2];
[1]Univ. of Illinois at Chicago, Univ. of Illinois at Chicago, Chicago, IL; [2]Sensory Motor Performance Prog, Rehabil Inst. of Chicago, CHICAGO, IL

Abstract: Recent studies have shown benefits of patient-initiated exploratory practice, yet the tools for interpreting adaptation in such movement require further attention. In a test on 10 chronic stroke subjects practicing for 3 days, we found that inter-quartile range of motion did not show improvement in movement range. However, distribution analysis using multivariate Gaussians revealed greater complexity of movement patterns by the end of training. Furthermore, linear discriminant classification analysis revealed that each movement distribution could be uniquely associated with each patient. Interestingly, this analysis indicated greater distinction in acceleration data compared to position or velocity. These results show that distribution analysis provides more powerful tools for detecting differences in movement deficits. The impact of this work could be in improving therapy by incorporating distribution analysis in the design of customized interventions.



(A) Contour plots of acceleration histograms (for two typical subjects) versus multivariate normal functions with 1, 2, and 5 components, reveal new movement patterns from Day-1 and Day-3 (Red/blue indicates greater/lesser observations). (B) Contrasts of histograms versus model functions indicate lower contrast with increasing components (columns), and higher contrast on Day-3 compared to Day-1. The color gradation (red/blue) indicates differences (greater/lesser) in the data compared to the models. These results show irregular changes in movement distribution across workspace.

Disclosures: J.L. Patton: None. **F.C. Huang:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.15/EEE16

Topic: D.17. Voluntary Movements

Support: Science Foundation Ireland 09/RFP/ECE2376

The Swartz Foundation (Old Field NY)

Title: Towards measurement of disordered motor networks in stroke using electroencephalography-based effective connectivity methods

Authors: *T. E. WARD[1,3], M. MIYAKOSHI[1], G. CRUZ[1,4], J. S. CHOE[2], K. SCHLICK[2], T. HEMMEN[2], S. MAKEIG[1];
[1]Swartz Ctr. for Computat. Neurosci., [2]UCSD Stroke Ctr., Univ. of California San Diego, San Diego, CA; [3]Electronic Engin., Natl. Univ. of Ireland Maynooth, Maynooth, Ireland; [4]Academic Unit of Mental Hlth. and Well-Being & Sch. of Psychology, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Stroke is the leading cause of physical disability and presents enormous societal and economic costs. Recovery, is driven by neuroplasticity - an intrinsic property of the brain that attempts to compensate for damaged motor networks. For about half of stroke survivors, recovery is not complete and the associated neurological symptoms, and the recovery dynamics, can be related to pathophysiological motor and compensatory networks that have arisen from the reorganization process. Recently, such networks have been monitored in vivo using connectivity analysis performed on functional MRI/PET data. The emerging consensus is that connectivity analysis will be key to our future understanding of stroke recovery. An especially exciting possibility is the application of this knowledge for the development of new, neurobiologically-informed treatment strategies. Current connectivity methods, however, are not suited to monitoring task-dependent networks during clinically relevant motor assessment tests, due to the practical constraints of MRI/PET. In addition, their intrinsically poor temporal resolution does not capture well, the transient changes in network coupling which are important for a comprehensive description of the motor program dynamics. To overcome the shortcomings of current approaches we propose a new methodology for simultaneous capture of brain and behavior using a combination of functional EEG neuroimaging and motion capture during standard clinical motor assessments. We present initial data collected from 128 channel EEG and full motion capture of the upper limb using a subset of the Wolf Motor Function Test with healthy subjects. We identified clear brain dynamics localized in the motor cortices which demonstrated beta suppression after onset of the right arm movement. The subsequent

multivariate vector auto-regressive analysis further revealed that the information flowed more from the left to the right hemisphere. The results demonstrate the feasibility of producing interpretable brain and behavior data during standard clinical assessments used to evaluate upper extremity motor deficits.

Disclosures: T.E. Ward: None. **M. Miyakoshi:** None. **G. Cruz:** None. **J.S. Choe:** None. **K. Schlick:** None. **T. Hemmen:** None. **S. Makeig:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.16/EEE17

Topic: D.17. Voluntary Movements

Support: Health Research Council of New Zealand 09/164R

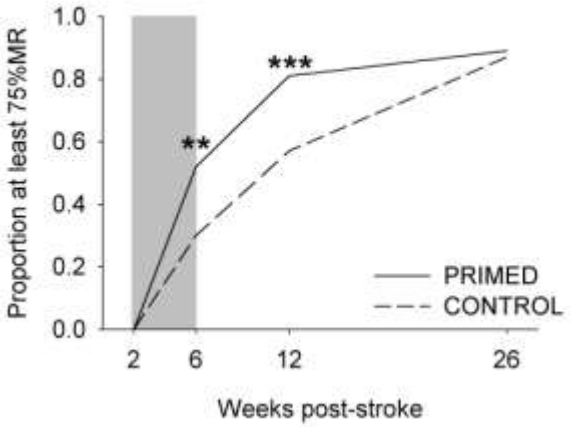
Stroke Foundation of New Zealand

Title: Bilateral priming accelerates recovery of upper limb function at the sub-acute stage after stroke

Authors: *W. D. BYBLOW[1], C. M. STINEAR[2], M. A. PETOE[2], P. A. BARBER[2], S. ANWAR[3];
[1]Ctr. for Brain Res. and Dept of Sport & Exercise Sci., [2]Ctr. for Brain Res. and Dept of Med., Univ. of Auckland, Auckland, New Zealand; [3]ADHB, Rehab Plus, Auckland, New Zealand

Abstract: The ability to live independently after stroke depends on the recovery of upper limb function. We hypothesised that bilateral priming with active-passive movements before upper limb physiotherapy would promote re-balancing of corticomotor excitability and accelerate upper limb recovery. A single-centre randomised controlled trial of priming with patients at the sub-acute stage after first-ever ischaemic stroke. The bilateral priming group made rhythmic mirror symmetric bimanual movements using a mechanical device for 15 minutes before undertaking 30 minutes of upper limb physiotherapy, every weekday for 4 weeks. The control group were given 15 minutes of intermittent cutaneous electrical stimulation of the paretic forearm before 30 minutes of physiotherapy. Measures of stroke severity, upper limb impairment and function were made at baseline, 6, 12 and 26 weeks. Transcranial magnetic stimulation was used to evaluate motor evoked potential measures in paretic and non-paretic wrist extensor muscles and MRI obtained to determine corticospinal tract integrity. The primary endpoint was relative % recovery of upper limb function at 12 weeks, measured with the Action Research Arm

Test. Intention to treat and per protocol analyses were conducted. Bilateral priming was suitable for 80% of patients who required upper limb therapy. Primed participants were 3 times more likely than controls to achieve at least 75% of their maximum recovery within 12 weeks, and a greater proportion of primed participants achieved at least 75% of their maximum score by 12 weeks (all $P < 0.05$). Primed participants exhibited greater re-balancing of corticomotor excitability than controls, evidenced by the slopes of stimulus-response curves at 12 and 26 weeks (both $P < 0.05$), and ipsilateral silent period at 26 weeks (both $P < 0.05$). Bilateral priming promoted neurophysiological reorganisation and accelerated recovery of upper limb function in the initial weeks after stroke. Further studies are required to determine if bilateral priming will increase the efficiency of rehabilitation.



Disclosures: W.D. Byblow: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Named Inventor on Patent for Training Device. **C.M. Stinear:** None. **P.A. Barber:** None. **M.A. Petoe:** None. **S. Anwar:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.17/EEE18

Topic: D.17. Voluntary Movements

Title: Simulated human arm motion drives perception-action learning in post-stroke individuals

Authors: *J. J. BUCHANAN[1], N. ROBSON[2], J. RAMOS[3];
[1]Texas A & M Univ., COLLEGE STA, TX; [2]Mechanical Engin., California State Univ., Fullerton, CA; [3]Mechanical Engin., Texas A&M Univ., College Station, TX

Abstract: Post-stroke individuals have varying degrees of hemiparesis in the arm contralateral to the lesion. This study was designed to evaluate if tracking computer simulated rhythmic arm actions can facilitate motor and visual perceptual learning in post-stroke individuals. Three individuals with varying degrees of hemiparesis that reduced elbow- wrist flexion-extension motion in the arm contralateral to the stroke participated. A visual perception rating task was given before and after motor training. The perceptual rating task required the individuals to rate 12 simulated elbow-wrist flexion-extension motion patterns. In the animation, the upper-arm remained stationary (forearm supine) and the sagittal plane elbow-wrist motion patterns were defined by 12 relative phase (ϕ) values (0°, ±30°, ±60°, ±90°, ±120°, ±150°, 180°), a constant elbow amplitude (92°), and a varying wrist amplitude (largest for $\phi = 0°$ and smallest for $\phi = 180°$). The simulated arm motions were rated as being easy (score 10) or hard (score 1) to produce. Participants did not move during the rating task. Between the pre- and post-training ratings, the individuals tracked animation patterns of $\phi = +60°$ and $\phi = +120°$ for three days with the arm contralateral to the stroke. Participants tracked the animation (12 cycles in 30 seconds) and concurrently received visual feedback in the form of a virtual stick figure of their arm. The level of hemiparesis influenced motor learning. Participant 1: characterized with mild hemiparesis demonstrated significant motor learning (in both ϕ and joint amplitudes). Participant 2: characterized by moderate to severe hemiparesis (wrist motion limited more than elbow) demonstrated motor learning in terms of upper-arm stability and elbow amplitude changes. Participant 3: characterized with severe hemiparesis (limited elbow and no wrist motion) did not demonstrate significant motor learning, yet responded to the animation. The level of hemiparesis influenced motor learning in the arm contralateral to the stroke. The visual motor training significantly impacted the visual perception of motor actions. Before training, the 0° and 180° patterns were rated as the easiest to produce and ±60° to ±120° were rated as the hardest. After training, the three participants rated the animated motions of ±60° to ±120° as easier to produce with ±90° rated as the easiest. The change in perceptual rating was similar to a group of younger adults that underwent similar motor training. In conclusion, simulated arm motion can serve as a visually relevant source of action information that can drive motor learning and in turn may enhance more traditional rehabilitation protocols.

Disclosures: J.J. Buchanan: None. **N. Robson:** None. **J. Ramos:** None.

Poster

563. Voluntary Motor Control: Stroke

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.18/EEE19

Topic: D.17. Voluntary Movements

Support: NIH/NIBIB POC-CENT U54-EB007954-01

Title: Robotic-assisted assessment of neurologic function in patients suffering from minor traumatic brain injury

Authors: *J. J. KORFHAGEN[1], J. M. MEUNIER[2], V. SUBBIAN[3], F. R. BEYETTE[3], G. J. SHAW[2];
[1]Neurosci. Grad. Program, [2]Emergency Med., [3]Sch. of Electronic and Computing Systems, Univ. of Cincinnati, Cincinnati, OH

Abstract: Introduction
About 1.2 million patients annually present to a U.S. Emergency Department (ED) with a minor traumatic brain injury (mTBI). Generally, these patients undergo a clinical neurological examination and a CT scan as part of their assessment. After ED discharge, 38-80% of these patients can experience chronic symptoms such as headache, fatigue, and difficulty concentrating; this is denoted ‘ postconcussive syndrome (PCS)’. Currently, there is no test to predict which mTBI patients develop PCS.
The Kinarm (BKIN Technologies Inc.) has been developed to utilize robotic technologies for objectively assessing neurologic function in humans and can detect subtle neurologic deficits not apparent in the standard neurological exam. The main goal of this project is to determine the relationship between ED-Kinarm scores and prevalence of PCS in mTBI patients. It is hypothesized that patients suffering from mTBI with abnormal Kinarm scores are more likely to develop PCS three weeks post-injury.
Methods
Patients are drawn from the population of a 90,000 visit per year Level 1 urban trauma center. Potential subjects are screened by clinical study assistants (CSAs) for clinical diagnosis of mTBI. Inclusion criteria include: Glasgow Coma Scale (GCS) score of 13-15, at least 18 years of age, blood-alcohol level lower than 100 mg/dl, and is physically capable of using the Kinarm device. The Kinarm device is composed of two robotic arms linked to a video screen and a computer interface. The computer controls the robots and monitors handle position. The screen displays interactive tests requiring the person to move the handles to complete a task. Five standard tasks measuring cognitive and sensorimotor function are used to determine neurologic status.
Three weeks post-injury, the patient completes the Rivermead Postconcussive Symptoms Questionare (RPQ) and Acute Medical Outcomes SF-36v2 Health Survey (SF-36) to determine if PCS is present.
Results
To date, 9 patients have been enrolled with 4 completing the study. Preliminary results show that 2 of these 4 patients have symptoms which may be consistent with PCS. Both of these patients

performed poorly on three of the five tasks while the other two performed poorly on one task.
Conclusion
Neurologic function of ED patients suffering from mTBI can be assessed using the Kinarm robot during their ED presentation. PCS at three weeks may be associated with worse Kinarm performance in these patients.

Disclosures: J.J. Korfhagen: None. **J.M. Meunier:** None. **V. Subbian:** None. **F.R. Beyette:** None. **G.J. Shaw:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.19/EEE20

Topic: D.17. Voluntary Movements

Support: Cerebral Palsy International Research Foundation

Lavin Pedriatic Fund

Foundation for PM&R

Title: Motor learning via low dimension remapping as a marker for spasticity and dystonia in cererbal palsy

Authors: *C. LOPEZ-ORTIZ[1], J. M. S. SIMKOWSKI[3], K. DOSHI[4], W. GOMEZ[5], D. GAEBLER-SPIRA[2];
[1]SMPP/PM&R, Rehabil. Inst. of Chicago/Northwestern Univ., CHICAGO, IL; [2]SMPP/PM&R, Rehabil. Inst. of Chicago/Northwestern Univ., Chicago, IL; [3]Biomed. Engin., Northewstern Univ., Chicago, IL; [4]Col. of Med., Univ. of Illinois, Chicago, IL; [5]Loyola Univ., Chicago, IL

Abstract: Children with cerebral palsy (CP) present atypical muscle tone and reduced selective motor control resulting in abnormal movements and postures. We tested a novel motor learning paradigm with feedback of reduced dimension in a virtual reality display as a marker for spasticity and dystonia in CP. All procedures were approved by the local IRB. 14 typically developing (TD) children and 14 age matched children with CP (mean age = 9.6 years, standard deviation (SD) = 1.4) and GMFCS I-II volunteered to participate in this study. 10 children participated in the spastic group (SG) and 4 children participated in the dystonic group (DG). Both groups completed the same training consisting of two matching games set to music. Both groups trained 3 times per week for 2 weeks and of 10-15min per game. Each child wore 8

reflective markers to track their movement with the NaturalPoint system at 100Hz (OptiTrack, Corvallis OR). The TD children performed a sequence of ballet postures requiring selective shoulder joint control in task 1 and additional hip joint control in task 2. Using statistical principal component analysis (PCA), the 24-dimensional space of the body trackables was reduced to a 2-dimensional space. The children in the SG and DG received continuous feedback about their own movement on a VR screen while aiming to match fixed target points from the TD group. After training, the children with both spastic and dystonic CP demonstrated motor learning by increasing the total number of matches with the targets from the first to the last training session in both tasks. Two-tailed t-tests showed a significant increase in the number of exact matches (alpha=0.05) for the SG (p=0.0019) and the DG (p=0.0433) in Task 1. In Task 2, significant improvement was seen in the spastic group (p=0.0015). Analysis of trajectory smoothness was computed using the spectral arc length metric based upon the Fourier magnitude spectrum of the movement speed. In Task 1, the group of children with spastic CP demonstrated significantly increased (alpha=0.05) smoothness of trajectory in 7 of the 8 trackables and in Task 2 for one trackable representing the right hand. The DG had no significant improvement in either task. Additionally, they had less trajectory smoothness by the last training session in Task 2. This presentation discusses the quantitative evaluation of selective motor control using classical ballet technique combined with low dimension remapping of body motions as quantitative marker to discriminate spastic and dystonic components in CP so as to optimize their clinical classification and therapeutic interventions.

Disclosures: C. Lopez-Ortiz: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cerebral Pasly International Research Foundation. **J.M.S. Simkowski:** None. **K. Doshi:** None. **W. Gomez:** None. **D. Gaebler-Spira:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.20/EEE21

Topic: D.17. Voluntary Movements

Support: CIHR operating grant

Title: Assessing cognitive-motor integration in preclinical Alzheimer's disease: A discriminant analysis and investigation of neural correlates

Authors: *K. M. HAWKINS[1], L. E. SERGIO[2];
[2]Kinesiology and Hlth. Sci., [1]York Univ., Toronto, ON, Canada

Abstract: The objectives of our research are 1) to characterize how the ability to integrate cognition into action is disrupted by Alzheimer's disease (AD) in its early stages and 2) to examine the neural correlates of impaired cognitive-motor integration in preclinical AD. We propose that measuring visuomotor integration under conditions that place demands on visual-spatial and cognitive-motor processing may provide an effective behavioural means for the early detection of underlying neuropathology. To this end, we have tested participants both with and without AD risk-factors on four visuomotor transformation tasks (standard, feedback reversal, plane dissociated, and plane dissociated + feedback reversal) using an Acer Iconia dual-touchscreen tablet. Comparisons between high AD risk participants (n=22) and both young (n=22) and old (n=22) healthy control groups have revealed significant performance disruptions in at-risk participants as task demands increase. A stepwise discriminant analysis was used to distinguish between high and low AD risk groups based on the outcome measures from our cognitive-motor integration assessment. The resulting classification accuracy was 86.4% (sensitivity: 81.8%, specificity: 90.9%).
We suggest that the impairments observed in high AD risk participants may reflect early neuropathology disrupting the intricate reciprocal communication between hippocampal, parietal, and frontal brain regions required to successfully prepare and update complex reaching movements. Currently, we are examining the underlying structural and functional connectivity in relation to AD risk and cognitive-motor integration performance in these participants. To date, seven at-risk participants and six age-matched controls have undergone anatomical, diffusion weighted, and resting-state functional connectivity scans. Preliminary analysis of these data has revealed significant correlations between peak activations in regions of the default mode network and task performance, as well as between the mean fractional anisotropy in white matter tracts and task performance. These data support our hypothesis that disruptions in cognitive-motor integration performance are associated with identifiable brain alterations.

Disclosures: K.M. Hawkins: None. **L.E. Sergio:** None.

Poster

563. **Voluntary Motor Control: Stroke**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 563.21/EEE22

Topic: D.17. Voluntary Movements

Support: NIH Grant R01 HD065438

Title: Modeling movement duration based on target-dependent differences to assess reaching movement recovery post-stroke

Authors: *H. PARK, S. KIM, J. GORDON, N. SCHWEIGHOFER;
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Abstract: According to the well known Fitts' law, distance and accuracy constraints (target size) affect reaching movement duration. In addition, the arm's biomechanics is known to influence duration, with movements in the direction of the largest inertia at the hand being the longest (Gordon et al. 1994). A possible third effect is that often practiced movements are faster (Keetch et al. 2005). We thus hypothesized that 1) movement distance, deviation from largest inertia, and the deviation from midline will predict reaching movement duration in healthy subjects, 2) reaching movements post-stroke will be longer in duration than those of healthy subjects and will depend on these three factors to a lesser extent, and 3) re-training will decrease movement duration and will increase the roles of these three factors in determining duration.
Six right handed non-disabled participants (2M, 4F, 50.2 ± 6.0 yrs) and five participants with stroke (3M, 2F, 59.8 ± 21.9 yrs, FM 42.4 ± 16.2; at least 1 year post-stroke) were asked to reach circular targets (diameter 3 cm) with the index fingers of their dominant or paretic hands as accurately and quickly as possible. The stroke group were asked to visit the laboratory for three consecutive days: on the first two days they received intensive training of 600 movements per day on 5 targets arrayed on an arc ranging from 30 to 150 degrees at 25 cm from the home position, as well as a pre- and a post-test. On the third day they received only a test. In each test, 35 targets were presented at different locations on a two-dimensional horizontal workspace arrayed from 30 to 150 degrees and at 10, 15, 20, 25, and 30 cm. The model of movement duration was fitted from data recorded in the tests, and is given by: $MD = a * \log2((2*D/size) + 1) + b * \cos(3*\pi/4 - q) + c * (1-\cos(\pi/2 - q)) + d$, where MD, D, size, and q represent the movement duration, the target distance from the starting position, the target size, and the target angle from the starting position in radians, respectively. a, b, c, and d are parameters to be estimated. Stepwise regression analysis was used for each test.
In the healthy group, mean movement duration was 290±60.0 ms and all parameters (a, b, and c) were statistically significant ($p < 0.02$) for all subjects, with a good average fit ($R2 = 0.73$). In the stroke group, movement duration decreased from 604±244 ms in the pre-test of day 1 to 458±154 ms in the post-test of day 2, but increased to 506±230 ms in the 24 hour retention test. Only distance accounted for the movement duration ($p < 0.009$) for all subjects in both group and all tests.

Disclosures: H. Park: None. **S. Kim:** None. **J. Gordon:** None. **N. Schweighofer:** None.**Poster**

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.01/EEE23

Topic: D.17. Voluntary Movements

Support: NIH Grant R01NS078311

Title: transcranial direct current stimulation for improving motor symptom in patients with Parkinson's disease

Authors: *Y. SALIMPOUR[1], Z. MARI[2], R. SHADMEHR[1];
[1]Dept. of Biomed. Engin., [2]Neurol. Dept., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Transcranial direct current stimulation (tDCS) is a promising noninvasive cortical stimulation method for adjunctive treatment of movement disorders. Here, we performed an experiment to quantify the effects of tDCS on PD motor symptoms. We considered an isometric task in which subjects held the handles of two robotic arms, one in each hand. The goal was to push on the left and the right robotic arms in order to move a cursor toward targets distributed around a circle. The position of the cursor was the sum of the force vectors produced by each arm. For each direction of target the subjects chose how much force to produce with their right and left arms. This choice was remarkably consistent over repeated days, suggesting that consistent cost dominated the choice of action. We hypothesized that the choice that people made was due to a cost that had two components: a cost for variability, and a cost for effort. To measure variability, in a unimanual task we measured variance of force for each arm at each direction of force. To measure effort, we measured the maximum voluntary force for each arm and each direction.We applied cathodal tDCS to M1 of the affected hemisphere and anodal in contralateral side of PD patients. This produced an immediate reduction in variance on the affected side in the unimanual task. That is, in PD cathodal stimulation of M1 reduced the variance for control of movements on the contralateral side. Consistent with this, the patients altered the choices that they made in the bimanual task: cathodal tDCS made their choices more similar to healthy controls. In contrast, the effect tDCS was very different in healthy controls: the reverse polarity (anodal M1 stimulation) of the electrodes in controls induced changes in bimanual behavior as we saw in PD patients with cathodal M1 stimulation. The laterality and flexion-extension indexes showed that PD patients were more responsive to tDCS in comparison to normal healthy controls. The results of applying tDCS in multi-session experiments also showed marked reduction in the motor subscore of the Unified Parkinson's Disease Rating Scale (UPDRS).In summary, we found that motor choice PD patients make is due to a cost analysis that has two components: variance, and effort. On the affected side, variance is significantly increased with respect to controls, and the choices of PD patients are generally consistent with this increased variance. We can improve unimanual variance of the PD patients using tDCS. This produces an immediate improvement in their actions in bimanual tasks. The repeated session of applying tDCS might improve motor symptom of Parkinson's Disease by decreasing the UPDRS and thus be therapeutically relevant.

Disclosures: Y. Salimpour: None. **R. Shadmehr:** None. **Z. Mari:** None.

Poster

564. Cortical Planning and Execution: Behavior

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.02/EEE24

Topic: D.17. Voluntary Movements

Support: NWO VICI Grant 453-08-004

Title: Relative proprioceptive distance is used for movements towards visual targets

Authors: *M. C. VAN DER GRAAFF, E. BRENNER, J. B. J. SMEETS;
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Abstract: When we want to make accurate hand movements to for instance a visual target, it is assumed that we combine visual and proprioceptive information to localize our hand precisely. It has been suggested that you do the same for visual targets. In this case, visual localization is based on the target's position relative to your fovea, and proprioceptive localization is relative to your hand. If you cannot see your hand, this proprioceptive target information will degrade, as updating the location of the target relative to your moving hand adds noise. If this proprioceptive estimate of the target is relevant, movements will become more precise and accurate if we are able to keep a proprioceptive estimate, for instance by placing the other (invisible) hand near the target. This unseen hand does not add new information; it only copies the visual information to proprioception. We tested this prediction by asking subjects to repeatedly move their right index finger between several visual targets on a table. We repeated the experiment after the subjects put their left index finger under one of the target locations (without any feedback). Subjects made considerable errors in positioning their invisible right finger. As predicted, adding a non-informative finger improved the accuracy of the movements (by about 20%). The bias of the invisible right hand after repetitive movements was on average shifted more towards the left hand's location than towards the target. When the invisible left hand was placed 10 cm to the right of the target, subjects also ended on average on a different place than the condition with no hand near the target. However now the endpoints of the invisible right hand did not simply shift in the direction of the left hand's location. This suggests that we are able to use a relative proprioceptive distance to localize a visual target.

Disclosures: M.C. Van Der Graaff: None. **E. Brenner:** None. **J.B.J. Smeets:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.03/EEE25

Topic: D.17. Voluntary Movements

Support: NIHR Stroke Association CQ7275

Title: The influence of affordances on object-directed actions

Authors: *E. ROUNIS[1,2], M. DUTA[2], G. HUMPHREYS[2];
[1]Oxford Univ., Oxford, Headington, United Kingdom; [2]Dept. of Exptl. Psychology, Cognitive Neuropsychology Ctr., Oxford, United Kingdom

Abstract: Studies in non-human primates suggest that the motor system selects which action to perform by a process of 'affordance competition'. This postulates that a number of potential actions may be represented simultaneously prior to one being selected when sufficient evidence accumulates to indicate that the action lead to a desired outcome (Cisek 2007).
We designed a forced-choice paradigm in which participants were required to use an everyday object (a mug) either with a congruent or incongruent hand grasp as specified by a line positioned to the top or the bottom of the mug. We varied both the orientation of the object (upright or upside down) and the action to be performed (based on a verbal instruction : 'lift' or 'turn'). To control for affordance effects elicited by the mug, participants also performed the same task on a cylinder with identical size and weight to the mug plus an identical line indicating an upright or rotated grasp.
The two tasks were presented in counterbalanced order across participants. Responses were characterized by four separate time stamps: 1) movement initiation; 2) movement duration (from movement initiation to lifting the object); 3) object handling duration (the time between the object lift and its repositioning) and 4) the return time (the time from re-positioning the object to the return to the resting position).
We found a strong effect of grasp orientation on movement initiation time and movement duration, both of which reduced for grasps to the top of an upright mug. It addition, the object handling duration reduced when the cup was returned into an upright position, suggesting an effect of completing a familiar action goal; in contrast return times were delayed when cups were transformed to an upright position.
This study demonstrates the dynamic influence of affordances and action goals on different parts of reaching, grasping and manipulating actions. A familiar affordance, from an upright cup, speeds action initiation and duration, while the subsequent affordance from a cup turned to

upright delays a return response. The presence of a familiar goal, to turn a cup to upright, facilitates enactment of the corresponding action.

Disclosures: E. Rounis: None. **M. Duta:** None. **G. Humphreys:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.04/EEE26

Topic: D.17. Voluntary Movements

Support: The study on the neural dynamics for understanding communication in terms of complex hetero systems, Grant-in-aid for scientific research on innovative areas, MEXT, Japan

Core Research of Evolutional Science & Technology, JST

Title: The influence of arm-movement preparation on interhemispheric beta synchronization in the medial motor areas

Authors: *T. NAKAJIMA[1,2], H. ARISAWA[1], R. HOSAKA[3,4], K. SHIMA[1], H. MUSHIAKE[1,2]; [1]Dept Physiol, Tohoku Univ. Sch. Med., Sendai, Japan; [2]Core Res. of Evolutional Sci. & Technol. (CREST), Tokyo, Japan; [3]Dept Appli Math, Fukuoka Univ., Fukuoka, Japan; [4]RIKEN Brain Sci. Inst., Wako, Japan

Abstract: The interhemispheric cooperation of the frontal lobes is of cardinal importance in controlling both bimanual and unimanual movements. In particular, the medial motor areas - the supplementary motor area (SMA) and the pre-SMA - seem to be pivotal regions to regulate the interhemispheric crosstalk via dense transcallosal connections. One of the measurements for the functional coupling between separate brain regions is the synchronized oscillation of field potentials. Although the beta wave (15~40 Hz) is a representative oscillation that is observed in motor-related areas including the SMA during motor preparation, its precise role concerning the interhemispheric cooperation is yet to be elucidated. To study how the beta oscillation mediates the functional coupling of the bilateral medial motor areas for the appropriate performance of manual movements, we trained monkeys to memorize and perform multiple bimanual or unimanual motor sequences. Bilateral recordings of local field potentials (LFPs) revealed significant level of interhemispheric phase synchrony in the beta band during motor preparation. We focused our analysis on unimanual sequences and found that the interhemispheric phase difference of the beta wave was modulated in relation to the intended arm use. Activity of more

than half of simultaneously recorded neurons was phase-locked to the beta oscillations, implying the presence of local generators of beta oscillations in each hemisphere. Our findings suggest that the direction of effective connectivity between the hemispheres changes in accordance with the prepared arm use and support the idea that the medial motor areas might inform the other hemisphere of intended movements.

Disclosures: T. Nakajima: None. **H. Arisawa:** None. **R. Hosaka:** None. **K. Shima:** None. **H. Mushiake:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.05/FFF1

Topic: D.17. Voluntary Movements

Support: a Grant-in-Aid for Scientific Research on Innovative Areas "The study on the neural dynamics for understanding communication in terms of complex hetero systems (No. 4103)" of MEXT, Japan

Title: Analysing a hand movement and gazes to find whether a monkey sets via points in a free curve drawing

Authors: *Z. HE, E. MYASHITA;
Dept. Computa. Intelligence & Systems Sci., Interdisciplinary Grad. Sch. Sci. &, Yokohama, Japan

Abstract: Detecting via points in a complicated movement can provide a clue to understand the brain mechanism of motor planning. It has been reported that drawing figures consisting of lines, e.g. triangles, the via point seems to be set to each vertex. However, there are few studies about via point setting when drawing a closed curve like a circle, which has no explicitly defined vertices. To find whether a monkey sets via points in free drawing of a circle-like closed curve, we investigated relationships between saccade end-points and a hand trajectory during the drawing. Two Japanese monkeys were trained to draw the circle-like closed curve manipulating a robotic arm manipulandum and watching a cursor on a computer monitor displayed as its hand position. The monkeys' hand positions were measured by rotary encoders within the manipulandum, and their gaze points were estimated using EyeLink (Monte System Corp., Japan). We tested the drawing with two different rotation directions and 8 different starting points, which were placed every 45° on a circle with a 100 mm-radius. First we compared a performance of the same monkey in each day and found that both monkeys showed a daily

learning process with a decrease of the variance of hand position although saccades occurred rather consistently. By analyzing data in a plateau period of the learning, we found that the variance of hand position was relatively smaller at points where the saccade occurred in higher probability compared with those where it occurred in lower probability. Finally we compared the saccade with the local minimum point of the variance of hand position in time axis and found that the saccade preceded the hand movement. The findings suggest that as the polygonal drawing, the monkey may set several via points in the curve drawing, where the monkey plan to pass through, and gaze the via points in advance to make sure that the hand is moved as expected.

Disclosures: Z. He: None. **E. Myashita:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.06/FFF2

Topic: D.17. Voluntary Movements

Support: Faculty Startup, A.L.Fantana

Title: Emergence of stereotypy in a complex movement sequence: A comparison of temporal and implementation variability in human motor learning

Authors: S. JETT, J. A. MEIER, J. KOSTEK, S. D. JAFFEE, *A. L. FANTANA;
Psychology Dept., Bowling Green State Univ., Bowling Green, OH

Abstract: Learned motor sequences are a foundation on which most human behavior is built. Skilled tasks as diverse as speaking, interacting with a computer, or playing a Bach cello sonata depend critically on the ability of the brain to activate the correct set of muscles at the correct time. Thus, an arbitrarily complex movement could be thought of as a string of motor acts triggered at specific times. One possible strategy for the brain to learn such a sequence would be to acquire these two aspects (temporal vs. gesture implementation) separately. Indeed, piano instruction often involves practicing individual finger movements separately before stringing them together and increasing the tempo to performance level. Alternatively, timing and implementation could be learned and improved on at the same time.
Here we present two longitudinal studies investigating the relationship between timing variability and movement structure variability in healthy adult volunteers learning a complex motor sequence. In both experiments, we follow the emergence of stereotyped behavior by accurately tracking movements using a high-speed (100-250Hz) and high-resolution (<1mm) 3-

D motion capture system. In the first experiment, we followed the finger movements in pianists with 15-25 years of experience over 3-6 weeks as they independently practiced a novel Beethoven prelude. We contrasted this self-timed behavior with an externally-guided sequence, a video game requiring precise movements, performed by undergraduate students with no musical training. In this experiment, the tempo of the sequence was held constant.
We found that the musicians were accurate throughout the study. Even when comparing early rehearsals, finger movements along the piano were stereotyped, both spatially ("correct notes") and temporally. While the overall tempo of the performance increased with practice, the coarse timing differences between renditions were distributed uniformly throughout the piece. However, when analyzing the detailed finger movement trajectories, we found a dramatic increase in stereotypy with practice: the motor implementation variability of the gestures decreased, and was mirrored by a corresponding reduction in temporal variability. The second experiment allowed us to separate the overall tempo from motor and uncorrelated timing errors. Here too, we observed a practice-induced parallel increase in temporal and motor precision.
Our results indicate that temporal and implementation variability show a similar time course during motor learning, whether in internally generated or externally guided movement sequences, suggesting a common neuronal origin.

Disclosures: S. Jett: None. **J.A. Meier:** None. **J. Kostek:** None. **S.D. Jaffee:** None. **A.L. Fantana:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.07/FFF3

Topic: D.17. Voluntary Movements

Support: R01 NS079664

Title: Reach-to-grasp: Widespread distribution in primary motor cortex of movement related potentials that vary with both reach location and object type

Authors: *A. T. ROUSSIN, A. G. ROUSE, M. H. SCHIEBER;
Neurobio. and Anat., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: In separate studies, reach direction and grasp type each have been shown to influence movement-related potentials (MRPs) in the primary motor cortex (M1). We investigated the spatiotemporal distribution of MRP variation with both reach location and grasp type. LFPs were recorded from 40 microelectrodes on 5 arrays chronically implanted in the anterior lip and bank

of the motor cortex as two rhesus monkeys performed a center-out, reach-to-grasp task. Electrode lengths ranged from 1.5 mm to 8.5 mm. Monkeys reached from a central object to grasp and operate one of four peripheral objects (sphere, button, handle, mallet) in a pseudorandom order. Each of the four objects required a different manipulation (rotate, press, pull, and pull, respectively). The locations of the objects were changed periodically, so that each object was presented at multiple locations, and multiple objects were presented at each location. Recorded LFPs were low-pass filtered, aligned at movement onset and then averaged across trials to obtain MRPs from each electrode for different combinations of reach location and object grasped.

MRPs typically were comprised of three prominent peaks: an initial positive peak between the instruction/go cue and movement onset (P1), a sharp negative peak around the time of object contact (N1), and a subsequent positive peak (P2). All three MRP peaks were widely distributed in M1. Two-way ANOVA for each of the three peaks showed that all three varied depending on both reach location and object type. Location and object effects both were present medially where ICMS evoked twitches in proximal muscles as well as laterally where ICMS evoked twitches in distal muscles.

In monkey L, object effects were stronger than location effects for all three peaks. In monkey X we found little if any P1. N1, which also was smaller in monkey X, showed stronger location effects than object effects. P2 in monkey X, as in monkey L, showed stronger effects of object than location.

M1 MRPs thus vary in relation to both reach location and object grasped. Both effects are widely distributed in the upper extremity representation.

Disclosures: A.T. Roussin: None. **A.G. Rouse:** None. **M.H. Schieber:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.08/FFF4

Topic: D.17. Voluntary Movements

Title: Left lateralized alpha power is associated with motor preparation of both hands

Authors: *T. KAJIHARA[1,2], M. ANWAR[1], Y. MIZUNO[1,3], M. KAWASAKI[1,4,5], K. NAKAZAWA[2], K. KITAJO[1,5,3];

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of Tsukuba, Tsukuba, Japan; [5]Lab. for Advanced Brain Signal Processing, RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: Covertly preparing a cued motor response decreases alpha-band (8-13Hz) electroencephalographic activity over the motor cortex. However, for the paradigm in which a cue indicates a subsequent imperative stimulus on differing probabilities, the alpha power differences between the contralateral and ipsilateral motor regions during the preparatory period remains unknown. Correcting the influences of visuospatial attention, the current study addresses the question using a variant of Posner paradigm (75% cue reliability). Right-handed participants were asked to covertly prepare a corresponding hand response (Left/Right/Non-Preparatory) based on a cue and execute it after the presentation of a target. Visual stimuli for the cue and the target were identical. The type of the cue did not significantly influence the change of alpha power activity over the motor region between the pre-cue and after-cue periods. However the cue per se, in other words regardless of the type of the cue, significantly decreased alpha power over the left motor region. Furthermore, for the after-cue period the alpha power of the left motor region was significantly lower compared to the right motor region across all the types of the cues. A previous study found, using a similar paradigm, showed that the lateralization of occipital alpha contralateral to visuospatial cue indexed visuospatial attention bias. On the contrary to our expectation, lateralization of motor preparation for one hand did not influence alpha activity over the motor-related region. Instead the preparation in general decreased alpha power in the left hemisphere. This result further consolidates the emerging view that motor-related function is rather localized to the left hemisphere for right-handed people.

Disclosures: T. Kajihara: None. **M. Anwar:** None. **Y. Mizuno:** None. **M. Kawasaki:** None. **K. Nakazawa:** None. **K. Kitajo:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.09/FFF5

Topic: D.17. Voluntary Movements

Support: NIH-R01-HD-045639

NSF-DMS0928587

AHA-11SDG7270001

Title: Implicit guidance to dynamic stability in rhythmic ball manipulation

Authors: *M. E. HUBER[1], D. STERNAD[2];
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Abstract: While rhythmically bouncing a ball with a racket is a seemingly simple task, it requires a high level of perceptually-guided coordination to be successful. In the experimental task, subjects manipulated a real table tennis racket to rhythmically bounce a virtual ball to a target height in a 2D virtual environment. Stability analyses of the model showed that dynamic stability is indicated when the racket contacts the ball during the decelerating portion of the racket's upward motion (Dijkstra et al. 2004). Dynamically stable performance implies that small errors converge back to stable performance without requiring active corrections, hence constitutes a "smart" solution that skilled performers adopt. The optimal racket acceleration at contact (RAC) for exploiting dynamic stability is approximately -3m/s^2.
The present study examined if a suitably designed perturbation could guide novice subjects negative RAC earlier in practice. The perturbation was applied to racket velocity at contact. Instead of using the actual racket velocity at contact, the calculation used the velocity value 50ms prior. When RAC was negative, the prior velocity was greater than the actual velocity, resulting in an increase in racket velocity at contact rendering higher ball amplitude for less velocity. When the RAC was positive, the reverse effect was obtained. We hypothesized that subjects learn to exploit dynamic stability faster by adding this perturbation (H1). We further hypothesized that when the perturbation is removed, subjects continue to exploit dynamic stability (H2).
Two groups of subjects performed the virtual ball bouncing task for 28 trials with each trial lasting 40 sec. The experimental group (n=6) practiced the task with the perturbation for 24 trials, followed by 4 trials under normal conditions. The control group (n=6) practiced the task without any perturbation. The objective in both conditions was to hit the ball rhythmically and accurately to the target height.
Results showed that with practice, both groups learned to reduce error between maximum ball height and the target line. However, there was a statistically significant difference in mean RAC between the two groups (p=.001). The experimental group learned to hit the ball with more negative RAC (H1). Removing the perturbation caused a statistically significant increase in mean RAC to -3.24m/s^2 in the experimental group (p=.001). This increase was towards the optimal RAC for exploiting dynamic stability of -3m/s^2 (H2). These results show that humans can be guided to exploit dynamic stability in coordinative behavior early in practice and maintain dynamic stability even when the guidance is removed.

Disclosures: M.E. Huber: None. **D. Sternad:** None.

Poster

564. Cortical Planning and Execution: Behavior

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.10/FFF6

Topic: D.17. Voluntary Movements

Support: NIH R01 NS065049

Title: Neural correlates of intrinsic and extrinsic coordinate control following left-hemisphere stroke

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Abstract: Previous research indicates that movements are controlled in both intrinsic (body-based) and extrinsic (world-based) coordinate frames. We have previously proposed that different motor tasks may preferentially rely on control in one frame or the other, and that these task differences may partially explain the pattern of intact and impaired motor abilities in stroke patients with ideomotor apraxia. Specifically, we proposed that many apraxia symptoms may be caused by disrupted intrinsic coordinate control (Buxbaum, 2001; Jax, Buxbaum, & Moll, 2006). In this study we tested 38 chronic left hemisphere stroke survivors and 11 age-matched controls on posture imitation and grasp imitation tasks designed to differ in their putative reliance on intrinsic (posture) or extrinsic (grasp) coordinate control. We also examined the relationship of performance on these tasks to performance on standard assessments of praxis and conceptual action knowledge, as well as the participants' lesion locations. Overall accuracy was lower for the stroke group than controls, and in both groups accuracy was lower for the intrinsic task than the extrinsic task. In addition, hand configuration accuracy differences between the intrinsic and extrinsic tasks were marginally larger for the stroke group than controls. A measure of performance on the intrinsic task relative to the extrinsic task (to control for overall severity) was significantly correlated with accuracy on pantomimed tool use and conceptual action knowledge. Finally, voxel-based lesion-symptom mapping analyses indicated that poorer-than-expected performance on the intrinsic task was associated with lesions to the superior portions of the posterior temporal lobe (BAs 21, 22, and 37) as well inferior portions of the angular gyrus (BA 39). These results provide further evidence that deficits in intrinsic coordinate control may be a core perceptual-motor processing deficit in ideomotor apraxia, and that these deficits may be localized to the left posterior temporal lobe and angular gyrus.

Disclosures: S. Jax: None. **D. Rosa-Leyra:** None. **L. Buxbaum:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.11/FFF7

Topic: D.17. Voluntary Movements

Support: NSERC Grant, Canada

Heart and Stroke Foundation of Canada

CIHR Group Grant ERRSM, Canada

Title: Referent control of motor actions by the corticospinal system in humans (TMS studies)

Authors: *A. G. FELDMAN[1,3], H. A. RAPTIS[2,3], N. ILMANE[2,3], S. G. SANGANI[4,3];
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Abstract: According to previous studies, muscles become active in response to deviations from a threshold (referent) position of body segments. Intentional motor actions result from central shifts in the referent position that represents the origin point of the spatial frame of reference in which these actions are produced. We tested the hypothesis that corticospinal pathways set and reset the referent position of body segments in a task-specific way without pre-determining movement kinematics or/and motor commands to muscles. Using TMS of the wrist motor cortex, we evaluated corticospinal influences at wrist positions established before and after voluntary motion. Such influences were also evaluated before and after involuntary motion elicited by sudden removal of a load (the unloading reflex). To minimize the effect of changes in motoneuronal excitability on the evaluation of corticospinal influences, motor potentials elicited by TMS were evoked during an EMG silent period produced by brief muscle shortening. Although the tonic EMG levels at pre- and post-unloading wrist positions were substantially different, the corticospinal influences remained the same. These influences changed however when subjects voluntarily moved their wrist to another position. An additional analysis showed that corticospinal influences shifted the referent position at which wrist muscles were recruited when voluntary wrist motion was made but that they maintained the same referent position during unloading. Thus, central control strategies underlying the two types of motor actions are fundamentally different. In another experiment, subjects learned to diminish the post-unloading movement extent (adjusted unloading). They were required not to activate antagonist muscles before unloading or make intentional movement corrections after unloading. We compared corticospinal influences before the adjusted unloading with those before the natural unloading. Tonic EMG activity levels of wrist muscles before the two types of unloading were similar. However, corticospinal facilitation of antagonist motoneurons but not motoneurons of pre-loaded agonist muscles was higher before adjusted unloading, suggesting that the referent position of the

joint was shifted by changing the sub-threshold state of the system in anticipation of unloading. The notion that the motor cortex may control motor actions by shifting spatial frames of reference for motor actions in a feed-forward way opens a new avenue in the analysis and understanding of brain function.

Disclosures: A.G. Feldman: None. **H.A. Raptis:** None. **N. Ilmane:** None. **S.G. Sangani:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.12/FFF8

Topic: D.17. Voluntary Movements

Support: A Grant from Yamaha Motor Foundation for Sports

Title: Oscillatory corticomuscular coupling is a negative factor of exerted force steadiness

Authors: *J. YAMADA[1], J. USHIYAMA[1,3], M. LIU[3], J. USHIBA[3,2];
[1]Grad. Sch. of Sci. and Technol., [2]Biosci. and Informatics, Keio Univ., Kanagawa, Japan;
[3]Rehabil. Med., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Oscillatory neural activity of the sensorimotor cortex shows coherence with contracting muscular activity within the 15-35 Hz frequency band (β-band) during weak to moderate intensity steady contraction (e.g. Baker et al., 1997; Conway et al., 1995). Our previous study demonstrated that the magnitude of coherence between electroencephalogram (EEG) over the sensorimotor cortex and electromyogram (EMG) varies among 100 healthy young individuals, and shows a significant positive correlation with the extent of the β-band oscillation in EMG (Ushiyama et al., 2011). Considering this result, it is hypothesized that the strength of corticomuscular coupling is a factor regulating the steadiness of individual motor output. To test this hypothesis, we recorded the EEG over the sensorimotor cortex and EMG from the tibialis anterior muscle, when the healthy participants (n = 18, aged 20-33 yrs) performed steady dorsiflexion at 30% of maximal effort for 60s. As results, individual magnitude of EEG-EMG coherence (peak coherence) showed significant positive correlations with the coefficient of variation of force and with the sum of the power spectral density of the force within β-band (Force β-PSD) (both, $p<0.01$). Furthermore, we divided lower frequency band of force signal into 5-14 Hz (Force High α-band) and 1-4 Hz (Force Low α-band) bands, based on some previous studies which have reported the existence of physiological tremor around 10 Hz during isometric contraction in lower limb (Elble and Randall, 1976; McAuley and Marsden, 2000). The magnitude of EEG-EMG coherence also showed significant positive correlation with Force High

α-PSD ($p<0.05$) but not with Force Low α-PSD. Next, we conducted further examinations to clarify the association between the β-band EMG oscillation and the High α-band force fluctuation. By careful visual observations, we found that the amplitude of EMG rhythmic burst fluctuated within the task performed by the participants with greater EEG-EMG coherence. Further, the rhythm of bursts and the duration of silent period between two successive EMG bursts changed randomly. In such cases, force fluctuations at around 10 Hz were characteristically observed in the force signal. By contrast, EMG signals in the participants with weaker EEG-EMG coherence showed tonic activation patterns, and its amplitude was stable throughout the task. Such muscle activation patterns would lead to more precise force output. Overall, the present data suggest that the β-band corticomuscular coupling is a determinant of individual muscle activation patterns, and therefore influences the force steadiness of each participant.

Disclosures: J. Yamada: None. **J. Ushiyama:** None. **M. Liu:** None. **J. Ushiba:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.13/FFF9

Topic: D.17. Voluntary Movements

Title: Decreased ability to inhibit motor response under the food craving condition

Authors: *K. YAMANAKA, F. ISHIKAWA, Y. FURUKAWA;
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Abstract: Human eating behavior is driven by their craving for foods and achieved with an action of reaching for foods. Therefore, inhibiting an action is difficult when human is craving for foods. However, it remains unclear how human ability to inhibit an action is influenced by the level of food appetite. Here, we compared the performance of Go/Stop task, in which participants need to inhibit a planned action, between conditions in which the level of food appetite was distinctly different: just before and after food intake. Twenty volunteers (12 females and 8 males) have conducted two experimental sessions on separate days. All participants were invited to laboratory between 11am and 2pm. They were previously instructed to have almost the same breakfast at almost the same time on both days. After the task instruction, they performed Go/Stop task, consisting of 100 Go and 100 Stop trials. They had almost the same lunch in the laboratory on both days, but the timing is different: one was before and the other was after Go/Stop task (pre-task and post-task conditions). Just before and after Go/Stop task, their ratings

of food appetite (desire to eat, hunger, and fullness) were assessed with 50-mm visual analog scales and their perceived levels of arousal, pleasant, excite, and stress were assessed with 9×9 affect grid. In the Go/Stop task, subjects were instructed to click a computer mouse by a right index finger when an indicator moving with a constant velocity reached a target (Go trial) or to avoid the click when the indicator randomly stopped 250-100 ms before it reached the target (Stop trial). From the %correct in Stop trials and mean response time in Go trials, stop-signal reaction time (SSRT) was calculated for each participant and condition. As expected, food appetites significantly decreased in post-task condition compared with pre-task condition. On the other hand, perceived levels of 4 items were not significantly different between pre- and post-task conditions due to their large between-subject variability. SSRTs tended to decrease in the post-task condition compared with the pre-task condition, but they were not significantly different. Therefore, analysis of covariance was conducted with condition as a main factor and each perceived levels as a covariate. As a result, only when perceived stress level was included as a covariate, SSRTs in the post-task condition were significantly smaller than those in the pre-task condition. These results indicate that, considering the effect of perceived stress levels, human motor inhibitory function might change before and after the food intake and suggest difficulty to inhibit a planned action under the food craving condition.

Disclosures: K. Yamanaka: None. **F. Ishikawa:** None. **Y. Furukawa:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.14/FFF10

Topic: D.17. Voluntary Movements

Support: Natural Sciences and Engineering Research Council of Canada

Title: Increased task complexity leads to a decreased motor preparatory state

Authors: *N. M. DRUMMOND, E. K. CRESSMAN, A. N. CARLSEN;
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Abstract: Previous research using a startling acoustic stimulus (SAS) has shown that highly practiced simple movements are prepared well in advance (1500 ms) of the earliest go-signal during a variable foreperiod simple RT task (Carlsen & Mackinnon, 2010). The purpose of the present experiments was to investigate the level of advance preparation in the motor system while participants completed a more complex task. Specifically, participants completed a visuomotor mental rotation (VMR) task within a variable foreperiod RT framework, requiring

them to perform different movements on each trial. A trial started with the appearance of a visual cue and an instructed angle of rotation (0°, 60°, 90° or 120°) 1650-2350 ms prior to the go-signal. Participants were then required to initiate their movements to the specified location (visual cue + angle of rotation) as quickly and accurately as possible following the go-signal. Instructed angles of rotation were presented either during random or blocked conditions, such that all angles were presented within a block of trials or only one angle was presented within a block of trials, respectively. To probe motor preparation a SAS was randomly presented 500, 1000, or 1500 ms following visual cue onset (i.e., 1150, 650, or 150 ms prior to the earliest go-signal). Results revealed that subjects were consistently and reliably startled at all three SAS time points during both random and blocked trial conditions. Interestingly, in comparison to previous tasks in which participants moved directly to the same target on all trials (Carlsen & Mackinnon, 2010), results revealed a reduced level of advance motor preparation in this more complex task for both random and blocked trial conditions, as indicated by the decreased proportion of movements involuntarily triggered by the SAS at all three time points. Furthermore, results indicated no differences in the proportion of SAS-triggered movements between random and blocked trial conditions or between any of the angles of rotation. These results suggest that the complexity of the task itself may be what influences the level of motor preparation achieved. More complex tasks and their resulting movements may introduce a larger amount of neural noise into the motor system than tasks involving a more simple movement. Increased noise may then result in a lower mean level of motor preparation in order to guard against accidental early and/or errorful response initiation. We postulate that these differences in preparatory state prior to the imperative go-signal between tasks are strategic, such that humans employ a preparatory strategy that accounts for noise introduced into the motor system by task demands.

Disclosures: N.M. Drummond: None. **E.K. Cressman:** None. **A.N. Carlsen:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.15/FFF11

Topic: D.17. Voluntary Movements

Support: FAPERJ E26/111.640/2011

Title: Adult hemispherectomy asymmetrically affects the emotional reactivity and locomotion activity in mice

Authors: *M. C. SANTOS[1], D. PAES BRANCO[2], Y. ABREU VILLAÇA[2], A. CHRISTIAN MANHÃES[3], C. CARNEIRO FILGUEIRAS[2];
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Abstract: Evidence exists indicating that cerebral lateralization is a fundamental feature of all vertebrates. In humans, it has been suggested that the right hemisphere is involved in processing negative emotional information and the left hemisphere is involved in control of motor function. In rodents, evidence for hemispheric lateralization is sparse. In this regard, here, we used unilateral hemispherectomy to study the relative importance of each hemisphere in controlling emotion and spontaneous motor activity in mice. Adult male mice were submitted to right hemispherectomy (RH), left hemispherectomy (LH) or sham surgery (SHAM). To help the interpretation of results an addition sample of mice was submitted to unilateral suction of left frontoparietal area (LFP), right frontoparietal area (RFP) or sham surgery (CONT). Fifteen days after surgery, both the emotional reactivity and the spontaneous locomotor activity were assessed in the open field test 10 min (divided into 1 min intervals). The open field arena consisted of a polypropylene box in which the floor was divided into 16 same-sized rectangles. The total number of rectangles crossed was used as the measure of spontaneous locomotor activity. Considering that mice avoid open areas, the ambulation in the center and time spent in the central rectangles were used to assess emotional reactivity. Regarding locomotor activity, the two surgical techniques reveal asymmetries in opposite direction. The locomotor activity of LH, which increased along the test period, was higher than both RH and SHAM. In contrast, the locomotor activity of RFP which decreased along the test period was higher than both LFP and CONT. Regarding emotional reactivity, the LH group spent less time in the central area than both RH and SHAM groups. No differences were observed between LFP, RFP and CONT groups. Our results suggest that the two hemispheres contribute asymmetrically to control of emotional reactivity and to control of motor activity in mice. Similarly to what is observed in humans, the right hemisphere of mice was more involved in processing negative emotional information. Regarding locomotor hyperactivity, the differences observed between hemispherectomized and frontoparietal-lesioned mice suggest that multiple lateralized circuits (or systems) may underlie spontaneous locomotor activity.

Disclosures: M.C. Santos: None. **D. Paes Branco:** None. **Y. Abreu Villaça:** None. **A. Christian Manhães:** None. **C. Carneiro Filgueiras:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.16/FFF12

Topic: D.17. Voluntary Movements

Support: NIH Grant MH55987

NIH Grant MH078824

Title: Social cues can acutely reverse injury-induced vocal motor impairments

Authors: *M. H. KAO, R. RAJAN, A. DOUPE;
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Abstract: Motor skills, such as speaking or typing, involve combining simple movements in novel sequences. Performance of such skills is influenced by external cues, especially when the brain is damaged by injury or disease, but the underlying mechanisms are poorly understood. Songbirds are an excellent model system for studying the generation of learned movements. In adult zebra finches, social cues strongly modulate motor output: song rate and stereotypy are greater when males produce female-directed courtship song than when they sing alone ('undirected'). Previous work has shown that microlesions of a premotor nucleus, HVC, immediately destabilize undirected song, disrupting its temporal and spectral organization. To examine whether social cues can reverse the effects of HVC damage and facilitate stereotyped song, we made partial lesions of HVC and examined the consequences on song in different contexts. We found that while lesioning 40-80% of HVC induced gross changes in the sequence and structure of song elements ('syllables') when males were alone, female presence could dramatically "repair" song. The timing, acoustic structure, and order of syllables during courtship song were similar to that of pre-lesion song, and birds with HVC lesions rapidly switched between variable, degraded undirected song and more stereotyped, mature courtship song. To examine neural mechanisms underlying the social modulation of song, we focused on a basal ganglia-thalamocortical loop known to mediate song variability in normal birds. We reversibly inactivated the cortical output (LMAN) of this loop while disrupting HVC activity, and found that LMAN inactivation prevented the destabilization of song normally induced by HVC disruption. These findings indicate that song disruption following HVC damage likely derives from aberrant activity in this loop, which can be mitigated by context. More generally, they suggest that external cues facilitate movements in patients with motor disorders by suppressing pathological activity in basal ganglia-thalamocortical loops.

Disclosures: M.H. Kao: None. **R. Rajan:** None. **A. Doupe:** None.

Poster

564. **Cortical Planning and Execution: Behavior**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 564.17/FFF13

Topic: D.17. Voluntary Movements

Support: NIH grant HD59983

NIH grant NS44393

NIH grant HD58301

Title: Frontal and parietal lesions have different effects on online updating of prehension for perturbations of object goal

Authors: E. TUNIK[1], S. T. GRAFTON[2], *S. V. ADAMOVICH[3];
[1]Rehabil. and Movement Sci., Rutgers Univ., Newark, NJ; [2]Dept. of Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA; [3]Dept of Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ

Abstract: It is now known form primate physiology studies that the region in the anterior portion of the intraparietal sulcus (area AIP) and the ventral premotor cortex (area F5, PMv) contain neurons whose firing rates are tuned to the features of object shape and to the hand shape used to grasp an object. Since aIPS and PMv both encode 3D features of objects and grasp shape, we set out to test the extent to which each region contributes to updating of the entire hand shape, rather than updating a single grasp parameter (which presumably can be achieved simply by a scaling solution). To test this, 8 healthy right-handed young adults observed a rectangular target object for 200ms before vision was blocked with liquid crystal glasses. The object was initially oriented such that the bottom edge was either parallel to the table or at a 45-degree angle (square and rhombus orientation, respectively). Following the initial view period, subjects were cued to reach-grasp the object such that the four fingers were either adducted and flexed together (square object) or abducted and less flexed (rhomboid object). At the start of movement, vision was restored again for another 200ms revealing to the subjects that the object orientation either remained unchanged or was perturbed to the other orientation (rhombus-to-square or square-to-rhombus), requiring them to either maintain the planned hand shape, or to update it. Double pulse TMS (50ms apart) was used to disrupt processing in aIPS and PMv either in the second viewing interval or immediately afterward, to tease apart processes related to updating versus execution. Subjects also performed the task with Sham TMS to the vertex as a control block. Kinematic analyses revealed that scaling of peak excursion of finger abduction and flexion was significantly affected and delayed when TMS was applied to PMv and aIPS at the updating interval, but not at the execution interval. Eleven joint angles submitted to linear discriminant analysis for classifying the hand shape identified a key difference between aIPS and PMv disruption on the evolution of hand shaping. aIPS-TMS led to a delay of hand shaping that

eventually recovered to the level of the Sham-TMS condition, without increasing movement time. Conversely, PMv-TMS led to a delay and an entirely different hand shaping pattern that was also associated with ~15% longer movement times. Our findings suggest that PMv and aIPS form unique but complementary nodes required for updating in the cortical reach-to-grasp network. Based on our findings, we speculate that aIPS may be integrating sensory information while PMv may be supplying a model of the grasp shape, which if disrupted must be rebuilt de novo.

Disclosures: E. Tunik: None. **S.T. Grafton:** None. **S.V. Adamovich:** None.**Poster**

565. **Sensorimotor Control in Orofacial Systems**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.01/FFF14

Topic: D.17. Voluntary Movements

Support: NIH Grant DC008149

NIH Grant DC005935

Title: Age-related changes in rat cortical motor area dedicated to tongue and jaw

Authors: J. M. WENNINGER[1], J. RUSSELL[1], H. KLETZIEN[1], A. J. SCHASER[1], J. A. KLEIM[2], N. THOMAS[2], *N. P. CONNOR[3];
[1]Surgery, Univ. of Wisconsin, MADISON, WI; [2]Arizona State Univ., Temple, AZ; [3]Univ. Wisconsin Med. Ch, MADISON, WI

Abstract: Age-related changes in sensorimotor actions for speech, voice, and swallowing may be due to alterations within cranial muscles, neuromuscular junctions (NMJ), and the central nervous system. Within the tongue and hypoglossal nucleus (HN) of an aging rat model, we have found reductions in evoked and voluntary muscle forces, alterations in NMJ morphology, alterations in serotonergic inputs to the HN, and a reduction in primary dendrites of hypoglossal motor neurons. However, it is unknown if the age-related decline in sensorimotor function involves cortical map changes.
To test the hypothesis that cortical motor areas dedicated to jaw and tongue would decrease as a function of aging, we performed intracortical microstimulation mapping experiments in young adult (9 mo, n=7) and old (36 mo, n=7) Fischer 344/Brown Norway male rats. Animals were anesthetized and placed in a stereotaxic frame and a cranial window was made extending 3-5mm rostral and 1-2mm caudal to Bregma. A photograph of the cortical surface was taken and a digital grid (squares of 250μm) was overlaid. Sites of stimulation at a depth of 1550 (±5) μm were made at grid intersections using a glass microelectrode filled with 3M NaCl and platinum

wire. Current was increased until movement was evoked (maximum stimulus 100μA), and a threshold stimulus for movement was determined. Mean areas of tongue and jaw movement were calculated.
We found that old rats had larger tongue movement representations than young adult rats (0.889±0.125 and 0.204±0.093 mm^2, respectively; p = 0.001). However, there was no difference in the size of jaw movement representations between old and young adult rats (1.08 ±0.334 and 0.666 ±0.104 mm^2, respectively; p = 0.28). Additionally, only 2 of the young adult rats had a tongue area greater than 0.06 mm^2. Thus, these results did not allow us to accept our hypothesis. While it appears that the cortical area dedicated to tongue increases with age in male rats, age-related cortical thinning may have occurred. Because stimulation was evoked at a constant cortical depth, further investigation of total cortical volume dedicated to the tongue is needed.

Disclosures: J.M. Wenninger: None. **H. Kletzien:** None. **A.J. Schaser:** None. **J.A. Kleim:** None. **N. Thomas:** None. **N.P. Connor:** None. **J. Russell:** None.

Poster

565. **Sensorimotor Control in Orofacial Systems**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.02/FFF15

Topic: D.17. Voluntary Movements

Support: R01DC012502

Title: Modulation of auditory and somatosensory event-related potentials due to speech motor learning

Authors: *T. ITO[1], J. H. COPPOLA[2], D. J. OSTRY[3,2];
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Abstract: The neural mechanisms of sensory plasticity associated with speech motor learning are little understood. In arm reaching motion, motor adaptation to novel external environment changes somatosensory function and its associated cortical processes as well as motor function. Since both auditory and somatosensory function are involved in speech production and learning, speech-related auditory and somatosensory cortical processes may possibly be modified in speech motor learning. We here examined whether speech motor learning alters auditory and somatosensory cortical processes by studying event-related potentials (ERPs). We tested native speakers of American English. We applied altered auditory feedback (AAF) training as a motor learning task. When subjects repeated aloud the speech utterance “head,” the produced sound

was played back through headphones. The first formant of /ea/ in “head” was gradually decreased over 50 repetitions and held at a maximum change for a further 110 repetitions. 9 of 18 participants showed a gradual increase of the first formant frequency (F1) in their utterance repetitions, an adaptive change in response to the experimentally imposed decrease of F1 in the sounds that were played back to them through headphones. In order to evaluate the effects of this adaptation on cortical sensory processes, we recorded auditory and somatosensory ERPs using 64-channel electroencephalography before and after AAF training. Auditory ERPs were elicited by using the synthesized vowel sound “e” corresponding to /ea/ in “head”. Somatosensory ERPs were elicited by facial skin deformation similar to that which occurs in the articulatory movements in the production of “head”. We found changes to auditory and somatosensory ERPs following AAF training in individuals who showed adaptation change to altered auditory feedback, but not in individuals who did not show an adaptation effect. Auditory ERPs in post training were significantly reduced at electrodes over the right frontal regions. The changes in ERPs were correlated with the amount of adaptation in AAF training. In somatosensory ERPs, we found an enhancement of the mu rhythms in individuals who showed adaptation at the C5 electrode over left sensorimotor area. The results demonstrate that speech motor learning modulates the cortical processing of speech-related somatosensory and auditory inputs.

Disclosures: T. Ito: None. **J.H. Coppola:** None. **D.J. Ostry:** None.

Poster

565. **Sensorimotor Control in Orofacial Systems**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.03/FFF16

Topic: D.17. Voluntary Movements

Support: NIH R01 NS045853

Brain Research Foundation

Title: Spatiotemporal dynamics of multiple bands of local field potentials from orofacial portion of primary motor cortex during feeding

Authors: *K. TAKAHASHI, J. IRIARTE-DIAZ, K. A. BROWN, N. G. HATSOPOULOS, C. F. ROSS;
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Abstract: The orofacial area of the primary motor cortex (MIo) is involved in control of voluntary, semiautomatic and rhythmic movements of the tongue and jaw during key phases of

feeding behaviors such as ingestion, chewing and swallowing. Our previous results showed that beta oscillations (15~25Hz) of local field potentials (LFPs) increased their power immediately before swallow following a preferred unilateral chew cycle, and two bands of gamma oscillations (45~55Hz and 65~85Hz respectively) increased their power around the time of transition from food manipulation to first chewing cycle. Furthermore, we have shown previously from the hand/arm area of the primary motor cortex that prominent beta oscillations were present across the recording area of 4 mm x 4 mm, and propagated as planar waves along a rostrocaudal axis at speeds ranging from 10 ~ 30 cm/sec. To investigate if MIo exhibits similar spatiotemporal dynamics in each of the frequency bands identified from our previous study, we analyzed LFPs recorded from a Utah array implanted in MIo on the left hemisphere in each of two macaque monkeys while the monkeys were performing a manual feeding task. Tongue and jaw kinematics were captured using videofluoroscopy and a 3D motion tracking system. Most of the channels showed strong beta power at a consistent frequency, while gamma oscillations exhibited wide variations in peak frequency across the cortical surface. Beta oscillations propagated as planar waves and propagated bidiretionally along a rostrocaudal axis with speed ranging from 15 ~ 35 cm/sec. As for the gamma oscillations, only lower frequency gamma band propagated as planar waves with a unimodal direction distribution oriented towards the central sulcus and a speed distribution with a mode around 60 cm/sec. These results indicate that both beta and low gamma oscillations are spatiotemporally organized and propagate as planar waves. The spatial variations in gamma oscillation frequency across MIo suggest that gamma oscillations may be related to more localized neural activity than beta oscillations.

Disclosures: K. Takahashi: None. **J. Iriarte-Diaz:** None. **K.A. Brown:** None. **N.G. Hatsopoulos:** None. **C.F. Ross:** None.

Poster

565. **Sensorimotor Control in Orofacial Systems**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.04/FFF17

Topic: D.17. Voluntary Movements

Title: Neuronal networks innervating the jaw-opening and jaw-closing muscles: A retrograde transneuronal tracing study with rabies virus in the rat

Authors: *N. HATANAKA[1], S. MIYACHI[2], A. NAMBU[1], M. TAKADA[3];
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Abstract: Higher-order neuron networks innervating the jaw-opening and jaw-closing muscles were examined in the rat brain by intramuscular inoculation of rabies virus (CVS-11 strain). The virus was inoculated into either the anterior belly of the digastric muscle or the masseter muscle. The survival period after the inoculation ranged from 84 hr to 120 hr with 12-hr steps. The virus was initially taken up by trigeminal motoneurons for the jaw-opening and jaw-closing muscles, and then propagated through retrograde transneuronal transfer. Five infection stages were considered based on neuronal labeling in subcortical and cortical structures. At stage 1, only the trigeminal motoneurons were labeled. At stage 2, premotor neurons that project directly to the trigeminal motoneurons were labeled in the medullary, pontine, and mesencephalic reticular formation, medial parabrachial nucleus, Kölliker-Fuse nucleus, and sensory trigeminal nuclei. At stage 3, neurons that project to the trigeminal motoneurons indirectly via the premotor neurons were labeled in the cerebral cortex, amygdala, hypothalamus, zona incerta, intralaminar thalamic nucei, entopeduncular nucleus, substantia nigra pars reticulata, deep cerebellar nuclei, central gray, superior colliculus, and red nucleus. At stage 4, neuronal labeling occurred in the cerebellar cortex (Purkinje cells), ventrolateral striatum, and globus pallidus. In most of the regions examined, the distribution patterns of these neurons labeled multisynaptically from the jaw-opening versus jaw-closing muscles were largely similar. There were some differences. For example, the dorsolateral portion of the striatum exclusively contained the neurons for the jaw-opening muscle. This suggests that the dorsolateral striatum may specifically be involved in jaw-opening behaviors.

Disclosures: N. Hatanaka: None. **S. Miyachi:** None. **A. Nambu:** None. **M. Takada:** None.

Poster

565. **Sensorimotor Control in Orofacial Systems**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.05/FFF18

Topic: D.17. Voluntary Movements

Support: CIHR Grant MOP-4918

Title: Spiking activity in the motor and sensory cortices differ during long-term learning

Authors: *F. I. ARCE[1], N. G. HATSOPOULOS[1], J. C. LEE[2], C. F. ROSS[1], B. J. SESSLE[2]; [1]Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Fac. of Dent., Univ. of Toronto, Toronto, ON, Canada

Abstract: The sensorimotor cortices have long been implicated in the acquisition of novel motor skills. However, it is not known whether learning-related changes differ between the motor and

sensory cortices. To address this issue, we used chronically implanted microelectrode arrays to track neuroplastic changes in the activity of simultaneously recorded neurons in the face somatosensory (SIo) and motor (MIo) cortices and compared the changes between the two areas. Over a period of 9-11 weeks, two naïve monkeys (Macaca mulatta) learned to protrude the tongue onto a strain gauge and apply isometric force at the level cued by target positions. The two monkeys exhibited similar learning rates early in training. As task parameters became stricter, the monkeys significantly differed in their performance level.
We analyzed single unit activity recorded from MIo and SIo of each monkey related to 2 epochs during a task trial, i.e. preparatory (0.5 s after target onset) and movement (0.5 s after force onset). Single units were deemed task-modulated when their firing rates during a hold period (0.5 s before target onset) differed significantly from firing rates during the preparatory or movement epoch (Paired t-Test, $p<0.01$). The proportion of MIo and SIo neurons that were task-modulated significantly increased over the training period (Binomial test, $p<0.05$). However, the degree of modulation observed in MIo neurons significantly decreased (ANOVA, $p<0.01$) but such a decrease was not apparent in SIo. In both monkeys, trial-by-trial variability of the neural activity in MIo during the preparatory epoch progressively decreased over time (ANOVA, $p<0.01$) and exhibited a higher rate of change compared to the trial-by-trial variability of the neural activity during the movement epoch. In SIo, the response variability reflected the monkeys' performance. The high-performing monkey exhibited progressive reduction in the response variability during the preparatory and movement epochs (ANOVA, $p<0.01$) while the low-performing monkey showed increased response variability (ANOVA, $p<0.01$). Finally, pairs of MIo and SIo neurons showed significant modulation of coherent activity (2-6 Hz) regardless of monkeys' performance level. For the population of neuronal pairs, the peak of the coherent activity of MIo neuronal pairs was observed 0.25 s before force onset and was significantly earlier than that of SIo which was observed at the time of force onset (t-Test, $p<0.01$). Overall the results show neuroplasticity of both MIo and SIo cortices during long-term learning and point to differences in the way MIo and SIo neurons may contribute to the control of movement parameters as learning progresses.

Disclosures: F.I. Arce: None. **N.G. Hatsopoulos:** None. **J.C. Lee:** None. **C.F. Ross:** None. **B.J. Sessle:** None.

Poster

565. Sensorimotor Control in Orofacial Systems

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.06/FFF19

Topic: D.17. Voluntary Movements

Support: CIHR MOP4918

University of Toronto Rosenstadt Funding

Title: Decreased face primary motor cortex (face-M1) excitability induced by noxious stimulation of the rat molar tooth pulp is dependent on the functional integrity of face-M1 astrocytes

Authors: *L. AWAMLEH[1], H. PUN[1], J. LEE[1], B. SESSLE[1], L. AVIVI-ARBER[1,2];
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Abstract: * Awamleh and Pun contributed equally
Acute dental pain is a common clinical occurrence that is often associated with altered sensory and motor orofacial functions. We have previously shown that application of the small-fiber excitant and inflammatory irritant mustard oil (MO) to the rat molar tooth pulp results in central sensitization of trigeminal medullary dorsal horn (MDH) (and thalamic) nociceptive neurons that can be modulated by MDH application of the astrocytic inhibitor methionine sulfoximine (MSO) (e.g. Chiang et al., J. Neuroscience, 2007). The objectives of the present study were to determine whether: 1) MO application to the rat molar tooth pulp also affects face-M1 excitability manifested as an altered intracortical microstimulation (ICMS) threshold required to evoke electromyographic (EMG) activity in the right anterior digastric (RAD) -a jaw-opening muscle-; and 2) MSO application to face-M1 can modulate the MO effect on face-M1 excitability. Under Ketamine general anaesthesia, the right maxillary first molar tooth pulp was exposed with a high speed dental drill, and EMG electrodes were implanted into the RAD of Sprague-Dawley male rats. Following surgical exposure of the left hemisphere, a microelectrode was positioned at a face-M1 site from which ICMS (35ms train, 12x0.2ms pulses, 333Hz) evoked low-threshold ($\leq$30μA) RAD EMG activity. This baseline stimulation threshold was monitored every 15 min for 30 min; then MO (n=24) or saline (n=17) was applied to the exposed molar tooth pulp and ICMS thresholds were monitored every 5 min for 15 min. MSO (0.1mM, n=9) or saline (n=7) was then applied to the left face-M1 and ICMS thresholds were monitored every 10 min for 180 min. Data were analyzed by repeated-measures ANOVA followed by post-hoc Bonferroni as appropriate ($p<0.05$). Within 15 min of MO (but not saline) pulp application, RAD ICMS thresholds increased significantly as compared to baseline (49.9%$\pm$5.7%, Mean$\pm$SEM; $p<0.001$). One hour following MSO (but not saline) application to face-M1, elevated RAD ICMS thresholds decreased considerably towards baseline levels (14.2%$\pm$4.5%; $p<0.05$). These novel findings suggest that acute inflammatory dental pain is associated with decreased face-M1 excitability that is dependent on the functional integrity of face-M1 astrocytes and may be related to the mechanisms by which acute dental pain is associated with limited jaw movements.

Disclosures: L. Awamleh: None. **H. Pun:** None. **B. Sessle:** None. **L. Avivi-Arber:** None. **J. Lee:** None.

Poster

565. **Sensorimotor Control in Orofacial Systems**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.07/FFF20

Topic: D.17. Voluntary Movements

Support: Michael J Fox Foundation

Title: Early and progressive oromotor and swallowing dysfunction in a PINK1 knock-out model of Parkinson Disease

Authors: *M. R. CIUCCI, L. M. GRANT, C. A. KELM-NELSON;
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Abstract: Parkinson disease (PD) is devastating to oropharyngeal swallowing. However, the onset, progression and neural correlates of PD-related dysphagia are poorly understood. To address this, we used a transgenic rat model of PD and hypothesized that oromotor and swallowing deficits would manifest early in the disease process, progress, and be related to pathologies in brain structures associated with cranial sensorimotor function.
Rats with homozygous and heterozygous knock-out (KO) of PINK1, and WT controls (n=48;) were studied. Bite force and timing characteristics were measured during consumption of a 7cm piece of pasta at 4 and 8 months. Lingual force and timing control was measured during a complex licking task at 2, 4, 6, and 8 months. Videofluoroscopy was used to evaluate swallow function at 4 and 8 months (analysis ongoing). Tissue analysis is ongoing for key neurotransmitters and alpha-synuclein aggregates in brainstem, basal ganglia, and cortical regions important to oromotor function and swallowing. To determine differences between groups for biting and lingual forces/timing variables, a repeated measures ANOVA and Fishers Least Significant Difference tests were used (alpha<0.05).
Results show that rats in the PD models demonstrate increased interbite intervals regardless of age [$F(3, 96)=16.74$, $p<0.01$]. Contrary to our hypothesis, homozygous KO rats had increased lingual forces compared to WT ($t(46)=5.24$, $p<0.001$) and heterozygous ($t(46)=3.358$,$p=0.003$) at all-time points. However, there was an age by genotype effect for timing (average force over time)[$F(6,99)=9.7$, $p<0.001$] that shows homozygous rats press harder for the first 30 seconds of the task, then forces decayed during the task, and this deficit worsens as a function of age. Overall results indicate that transgenic PD rats, especially with homozygous KO of PINK1, demonstrate early oromotor and swallowing dysfunction, and that lingual deficits reflecting fine control and timing of licking behavior is progressively impaired. Full data sets will be presented for functional swallowing and pathology. Defining pathophysiology related to swallow deficits is essential to develop new treatment targets for PD-related dysphagia.

Disclosures: M.R. Ciucci: None. **L.M. Grant:** None. **C.A. Kelm-Nelson:** None.

Poster

565. Sensorimotor Control in Orofacial Systems

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.08/FFF21

Topic: D.17. Voluntary Movements

Support: NIH R01 DC009980

NIH F30 DE021944

Title: Kinematics of the pharyngeal swallow following palatal anesthesia in infant pigs: Evidence of motor learning

Authors: *R. Z. GERMAN[1], S. HOLMAN[2];
[1]Anat. and Neurobio., NEOMED, Rootstown, OH; [2]Sch. of Dent., Univ. of Maryland, Baltimore, MD

Abstract: Adaptive motor learning during swallowing occurs in adults, but its existence or prevalence in infants is unknown. A reduction in palatal sensation due to local anesthesia (PLA) injection is known to produce an increase in both hyoid elevation and jaw opening during swallowing. In some animals (Group A), there were no significant differences in kinematics after PLA. In others, however, the response was a distinct qualitative and quantitative change in hyoid, jaw and tongue kinematics (Group B). We examined the potential for motor learning or a feed forward mechanism during infant feeding using the Group B animals and measuring the time course of the swallowing motor response to palatal anesthesia. If motor learning occurred in the hyoid muscles, we would see a return to baseline hyoid elevation over time in the first feeding, and overshoot when the anesthetic wore off in subsequent feedings. On day one four infant pigs were fed during two sessions two hours apart that lasted 15-30 seconds and recorded with lateral videofluroscopy to assess normal kinematics. On day tow they were given palatal anesthesia and fed every 20 minutes for 15-30 seconds until normal suckling was observed. Over the course of each feeding session we measured hyoid and mandibular movements as well as airway protection with the Infant Mammal Penetration/Aspiration Scale. In all four pigs 30 minutes after PLA there was abnormal feeding, characterized by significantly more hyoid elevation and jaw opening, with no signs of returning to baseline levels, or adaptive learning. In three of four pigs, when normal sucking, characterized by normal jaw movement, returned 50-90 minutes after the anesthesia injection, the hyoid remained elevated early in the feeding and decreased over time. Jaw opening, however, immediately returned to baseline levels. This is the

first evidence that infant mammalian swallowing may also be capable of motor learning. Such evidence may provide the basis for the development of strategies for pediatric rehabilitation of dysphagia.

Disclosures: R.Z. German: None. **S. Holman:** None.

Poster

565. Sensorimotor Control in Orofacial Systems

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.09/FFF22

Topic: D.17. Voluntary Movements

Support: NIDCD 1R03DC012123-01

Title: Effects of targeted strength training in a unilateral vs. bilateral 6-hydroxydopmine model of Parkinson's disease

Authors: *E. K. PLOWMAN, B. RIVERA, K. DONOGHUE;
Communication Sci. and Disorders, Univ. of South Florida, Tampa, FL

Abstract: Background: Recent evidence suggests that motor training may be beneficial for slowing the onset of motor impairments in Parksinson's disease (PD). Aims: The aims of the current investigation were to: 1) determine the differential effects of unilateral versus bilateral striatal dopamine depletion on oral motor and limb motor function and 2) Determine the differential responses of oral and limb motor function in PD to targeted motor rehabilitation. Methods: Thirty-six male Long Evans were randomized to either a control (n=12), unilateral 6-OHDA (n=12) or bilateral 6-OHDA (n=12) group. Baseline performance of limb (reaching) and oral (licking) motor function were established prior to and six-weeks following intrastriatal 6-Hydroxydopamine (6-OHDA) infusions. Animals were then randomized to be in either a progressive forelimb strength training group or a progressive lingual resistance training group (n=6 in each rehab group) and underwent daily resistance training of either the tongue or forelimb. Following rehabilitation, motor performance was reassessed for a third and final time. High liquid protein chromatography was performed to determine levels of striatal dopamine depletion. Results: Both unilateral and bilateral 6-OHDA groups demonstrated significant reductions in lingual and forelimb force post-lesion ($p<0.05$). Progressive lingual resistance training had a significant treatment effect for bilateral 6-OHDA animals with noted increases in lick force following treatment. Unilateral 6-OHDA animals, however did not show improvements in lick-force impairments following eight-weeks of progressive lingual resistance training. A significant increase in forelimb force was noted for both the control and bilateral 6-

OHDA animals undergoing progressive forelimb strength training, however unilateral 6-OHDA animals did not improve forelimb force impairments post strength training. Conclusions: These data show that in a bilateral rodent model of PD, progressive strength training of both limb and oral motor function is effective for improving motor impairments.

Disclosures: E.K. Plowman: None. **B. Rivera:** None. **K. Donoghue:** None.

Poster

565. **Sensorimotor Control in Orofacial Systems**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 565.10/FFF23

Topic: D.17. Voluntary Movements

Support: CIHR MOP4918

University of Toronto Rosenstadt Funding

Title: Noxious tooth pulp stimulation decreases rat face primary motor cortex (Face-M1) excitability by modulating medullary astrocyte function

Authors: *H. PUN[1], L. AWAMLEH[1], L. AVIVI-ARBER[1,2], B. SESSLE[1];
[1]Oral Physiol., [2]Prosthodontics, Fac. of Dent. Univ. of Toronto, Toronto, ON, Canada

Abstract: H.P. and L.A. contributed equally to this work.
Dental pain is a common symptom forcing patients to seek dental or medical treatment, and is often associated with disruption of orofacial motor function. Application of the inflammatory irritant mustard oil (MO) to the molar tooth pulp in rats has been shown to induce central sensitization in medullary dorsal horn (MDH) nociceptive neurons that is dependent on MDH astrocyte function (e.g. Chiang et al., 2007). We hypothesized that such noxious stimulation of the tooth pulp would also result in altered face-M1 excitability that is dependent on the functional integrity of medullary astrocytes. To test our hypothesis, we examined if MO application to the rat molar tooth pulp affects face-M1 excitability manifested as an altered intracortical microstimulation (ICMS) threshold required to evoke electromyographic (EMG) activity in the right anterior digastric (RAD), a jaw-opening muscle, and if application of an astrocyte inhibitor (methionine sulfoximine, MSO) to MDH can modulate the MO effect. Under ketamine general anaesthesia, male Sprague-Dawley rats were implanted with EMG electrodes in the RAD. A high-speed dental drill was used to expose the right maxillary first molar tooth pulp. Following a craniotomy and cervical laminectomy, a microelectrode was positioned in the left face-M1 (<2.4mm depth) at a site from which ICMS (35ms train, 12x0.2ms pulses, 333Hz)

evoked low-threshold (≤30μA) RAD EMG responses. Baseline ICMS thresholds were monitored for 30 min, then MO (n=26) or vehicle (n=17) was applied to the tooth pulp. In some of these rats, 0.1 mM MSO (n=8) or vehicle (n=5) was applied to the MDH 15 min after MO application to the pulp. ICMS thresholds were monitored every 10 min for 200 min after MO or vehicle pulpal application. Changes in ICMS thresholds were analyzed by repeated-measures ANOVA followed by post-hoc Bonferroni-adjusted pairwise comparisons as appropriate, and are reported as mean ± standard error ($p<0.05$). RAD ICMS thresholds significantly increased within 15 min of MO pulp application (49.9% ± 5.7%, $p<0.001$) compared to vehicle application or baseline levels. However, within 15 min of MDH application of MSO, but not vehicle, the MO-induced elevated RAD ICMS thresholds decreased significantly towards baseline thresholds (9.7% ± 5.2%, $p<0.02$). Our findings suggest that motor disturbances arising from dental pain may involve decreased face-M1 excitability that is modulated by MDH astrocytes.

Disclosures: H. Pun: None. **L. Awamleh:** None. **L. Avivi-Arber:** None. **B. Sessle:** None.**Poster**

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.01/FFF24

Topic: D.17. Voluntary Movements

Support: FAPESP/BRAZIL

CAPES/BRAZIL

CNPq/BRAZIL

NAPNA-USP/BRAZIL

Title: Exercise promotes differential plastic effects in motor areas of the aged rat brain

Authors: J. BORBOREMA[1], S. SALAME[1], P. C. GARCIA[2], C. C. REAL[2], L. R. G. BRITTO[2], *R. S. PIRES[3];
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Abstract: Age-related changes in the central nervous system may generate cognitive and motor decline, such as reduction of memory, learning, balance, gait and complex motor skill. These deficits can be mitigated with physical exercise. However, little is known about the mechanisms involved in this neuroplastic improvement. The aim of this study was to analyze the expression

of the structural proteins microtubule-associate protein 2 (MAP2) and low molecular weight neurofilament (NF68), and synaptic proteins synapsin I (SYS) and synaptophysin (SYP) in the motor cortex, striatum and cerebellum of aged rats submitted to treadmill exercise (TE) and acrobatic exercise (AE). We used aged (18-month-old) male Wistar rats, divided into 3 groups: control-sedentary (SED, n=11), treadmill exercise (TE, n=13) and acrobatic exercise (AE, n=15). In the TE group, the rats were trained on a treadmill with maximum speed of 0.5 Km/h for 40 minutes, 3 times per week for 4 weeks. In the AE group, the rats went through circuits composed of various obstacles during the same period of time that the TE were trained. After 4 weeks of training, analyses of protein expression were carried out by immunoblotting and immunohistochemistry, and data were submitted to statistical analysis using ANOVA and Tukey post-test when appropriate. The level of significance was $p<0.05$. Immunoblotting and immunohistochemistry both revealed that trained animals, regardless of the type of exercise, showed distinct changes in structural and synaptic protein expression in the areas studied. AE and TE groups showed significant increase of MAP2, NF68 and SYS, with a decrease of SYP in the motor cortex, but the changes for the AE were bigger than those for TE, and were found with both techniques. In the striatum, both AE and TE generated an increase of NFs and SYP, with a significant decrease of MAP2 and SYS revealed by immunohistochemical data. The molecular and granular layers of the cerebellum responded with an increase of MAP2 and a decrease of NFs, SYS and SYP immunostaining. Immunoblotting data were unchanged after either AE and TE. These data suggest that the motor cortex of aged rats presents larger plastic responses than the other structures studied, and which were more evident after AE than TE, suggesting a larger participation of the motor cortex in neuroplasticity.

Disclosures: J. Borborema: None. **S. Salame:** None. **P.C. Garcia:** None. **C.C. Real:** None. **L.R.G. Britto:** None. **R.S. Pires:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.02/FFF25

Topic: D.17. Voluntary Movements

Title: Thenar cortical representation in humans associated with cellular-phone texting ability

Authors: *Z. A. RILEY[1], N. HOSEINI[2], J. MAO[2], N. R. ECKERT[2];
[1]Kinesiology, Indiana University- Purdue Univ. Indianapolis, Indianapolis, IN; [2]Indiana Univ., Bloomington, IN

Abstract: There is re-organization with motor learning that results in a change in the topographic motor 'map' or the distribution and connectivity of M1 neurons where representative areas expand or contract. These mechanisms enable the nervous system to adapt and consolidate practice of a task to improve performance. A novel way of studying motor skill learning and associated M1 plasticity is with cellular phone text messaging, where in the United States alone ~68% cell phone owners use texting as a means of communicating (mobiThinking.org). The purpose of the present study was to investigate changes in the motor cortical organization of a thenar muscle with transcranial magnetic stimulation (TMS) in individuals relative to their performance of a functional texting task (FTT) on a cellular phone keyboard. Stimulation-induced motor evoked potentials (MEPs) were examined in eight healthy subjects (6 males; range: 20-34 yrs) before and after a functional texting task (FTT). The FTT consisted of completing as many 45-character long phrases as possible in 2-minutes and served as a baseline measurement of the subject's texting ability. After the FTT the subjects were taken to an adjoining room and were seated comfortably in an upright position with the right arm resting on a padded table and placed in 60° shoulder abduction and 90° elbow flexion. TMS was used to stimulate an area over the left motor cortex corresponding to the representation for the right APB muscle, serving to map the motor cortex excitability. Subjects then completed a 10-minute practice FTT and finally the motor mapping procedures were repeated to examine any short-term changes that may have occurred with practice of the FTT. Texting score, indicating texting proficiency, was negatively correlated with the normalized map volume of the APB ($r = -0.78$, $p = 0.022$) and number of active stimulation sites ($r = -0.83$, $p = 0.009$). With texting practice there was a negative correlation between the subject's original texting score and the change in the Center-of-Gravity (CoG) ($r = -0.81$, $p = 0.014$). The results suggest there is an increased efficacy of existing synaptic connections in individuals that frequently use text messaging. It can also be demonstrated that a short-term adaptation to practicing a texting task is to move the CoG by 'unmasking' intracortical circuits. The biomedical significance of repeated cellular phone texting may serve in a manner to promote recovery of thumb control and dexterity, for example in stroke patients, in a manner similar to virtual reality gaming simulations.

Disclosures: Z.A. Riley: None. **N. Hoseini:** None. **J. Mao:** None. **N.R. Eckert:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.03/FFF26

Topic: D.17. Voluntary Movements

Support: CIHR

Title: Influence of BDNF polymorphism on I-wave TMS (ITMS) plasticity and neurophysiology in human motor cortex

Authors: *R. CASH, K. UDUPA, R. CHEN;
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Abstract: INTRODUCTION:
The influence of genetic variation in the brain-derived neurotrophic factor gene (BDNF) on human cortical neuroplasticity has recently attracted much interest. Motor cortical plasticity can be probed using repetitive TMS protocols including I-wave TMS (ITMS). Bidirectional modulation of cortical plasticity is possible with ITMS: ITMS(+) at ~1.5ms leads to LTP-like effects while ITMS(-) at ~2ms leads to LTD-like effects, attributed to phase-dependent plasticity mechanisms (PDP; Cash et al, 2012).
In the present study, we investigated the influence of single nucleotide polymorphism (SNP) BDNF Val66Met on ITMS plasticity, and additionally compared this with previously reported influence on Paired Associative Stimulation (PAS) plasticity. We also investigated the influence of ITMS (and genetic variation) on cortical inhibition neurophysiology, and compared this with PAS effects.
METHODS:
Ten subjects have thus far been studied: 5 Val homozygotes and 5 Met allele carriers. ITMS was optimized to individual I(1)-wave peaks and troughs for LTP-like ITMS(+) and LTD-like ITMS(-) protocols respectively (Sewerin et al, 2011). PAS(+) was likewise optimized to individual N20 latency and delivered at N20+2ms (Ziemann et al, 2004). Unconditioned single pulse motor evoked potential amplitude (MEP) and short and long interval intracortical inhibition (SICI and LICI respectively) were measured pre-and post-intervention.
RESULTS:
As anticipated, group results indicated that ITMS(+) led to an increase in MEP amplitude of ~150% post-intervention while ITMS(-) led to a reduction of MEP amplitude of ~20% compared to pre-intervention baseline. These changes were sustained for at least 30 minutes before returning to baseline between 45-60 minutes. ITMS(-) effects on MEP amplitude, SICI and LICI were more sensitive to BDNF genotype than ITMS(+), with Met allele carriers demonstrating reduced plasticity. PAS(+) effects were also greater in Val homozygotes.
DISCUSSION:
ITMS(-) LTD-like plasticity is more sensitive to BDNF Val66Met SNP than ITMS(+) LTP-like plasticity. This is consistent with the finding from cellular studies that LTD-like PDP is less robust than LTP-like PDP, and thus likely to be more sensitive to internal drivers of plasticity. The results furthermore suggest that ITMS optimized to individual I-wave latency may considerably increase the duration of ITMS after effects. Influences on inhibition will be discussed.

Disclosures: R. Cash: None. **K. Udupa:** None. **R. Chen:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.04/GGG1

Topic: D.17. Voluntary Movements

Support: Parkinson's Disease, UK

Medical Research Council, UK

Oxford Biomedical Research Centre

Title: Visuomotor adaptation and retention in the young, elderly and people with Parkinson's disease

Authors: M. PANOUILLÈRES, R. JOUNDI, J.-S. BRITTAIN, P. BROWN, *N. JENKINSON;
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Abstract: Motor adaptation is an essential process for the acquisition and refinement of motor skills and, importantly, for the recovery of motor function in neurological disease or after injury. Adaptation relies on plastic mechanisms in the brain which are known to be less efficient in healthy elderly people compared to young individuals. Transcranial direct current stimulation (TDCS) can improve motor learning and shows promise as an adjunctive therapy for motor rehabilitation. In this study, we explored the effects of TDCS on motor learning in healthy young and elderly subjects and in patients with Parkinson's disease (PD). Specifically, we measured visuomotor adaptation and retention of the adapted state after subjects received either sham or anodal TDCS over the primary motor cortex during adaptation. The task involved using a joystick-controlled cursor to follow a target that jumped from the centre of a computer screen to one of 8 locations situated concentrically about the screen. After an initial baseline phase where the relationship between the joystick and cursor was veridical, a visuomotor adaptation phase started, in which, the cursor movements were rotated counter-clockwise by 60° relative to the joystick movements. Subjects quickly adapted to the rotation and rapidly reduced the error between the cursor and the target over successive trials. Following the adaptation phase, subjects rested for 50 minutes. They then performed the task again with the same cursor rotation (60°), to assess short-term retention of the adaptation. The session ended with a washout phase, in which the movements of the cursor and the joystick were restored to normal. In this phase, subjects

started with large errors in the clockwise (opposite) direction but again quickly adapted back to the veridical joystick relationship. People with PD showed a similar ability to adapt to the visuomotor rotation as healthy aged controls, though the extent of adaptation in both elderly and PD groups was lower than that seen in young adults. PD patients showed impaired short-term retention compared to the elderly group and the young adults, who demonstrated the best retention. TDCS improved adaptation in all groups of participants. It also improved short-term retention, but only in elderly participants and people with PD. In sum, TDCS applied over M1 during visuomotor adaptation improved the ability of elderly and PD subjects to adapt and improved their subsequent short-term retention of the adapted state. Retention was not altered in young adults. Our results suggest TDCS may be an effective adjunctive tool for enhancing motor plasticity in patients with PD and the elderly.

Disclosures: M. Panouillères: None. **N. Jenkinson:** None. **P. Brown:** None. **J. Brittain:** None. **R. Joundi:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.05/GGG2

Topic: D.17. Voluntary Movements

Support: James S. McDonnell Foundation (Scholar award)

C7 - Cerebellar-Cortical

Title: Training, transfer and tDCS: Are training and tDCS effects generalizable, or effector and sequence specific?

Authors: *G. PRICHARD, C. VAROTSIS, S. WATERS-METENIER, J. DIEDRICHSEN; Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom

Abstract: Training on finger sequence tasks leads to a decrease in the time taken to complete a sequence at a comparable error rate. Whether and how this skill gain generalizes across effectors is uncertain. For sequences, there are multiple possible ways a learnt sequence could show inter-manual transfer of skill: extrinsically, according to an external reference frame (e.g. the untrained hand pressing the same keys on a piano keyboard, achieved by making non-symmetrical muscle movements), or intrinsically, according to an internal reference frame (e.g. the untrained hand making mirror-symmetric movements, but pressing different keys on the piano). Alternatively, skill gains could benefit the other hand for any sequence, whether learnt or novel. We examined

whether and how the brain transfers learnt skills to new effectors. Furthermore, we applied bihemispheric transcranial direct current stimulation (tDCS), known to improve learning, in order to assess how well the motor learning gains from brain stimulation generalize. Two groups (sham, tDCS) learned a set of sequences on the left hand for four days. Participants were then tested for two days with learnt sequences on the both hands, both in extrinsic and intrinsic reference frames, and on novel sequences. A further control group performed sequences on both hands for two days without training or tDCS. Both groups who received training performed better on the untrained hand than the group without training, however this effect was not specific to the intrinsically or extrinsically transformed sequences. tDCS improved the execution of the learnt sequences on the trained hand, and this effect generalized across hands, as well as to novel sequences. The representation of specific sequences does not appear to transfer across effectors, however training does result in more general task improvements, which are further enhanced by tDCS.

Disclosures: G. Prichard: None. **C. Varotsis:** None. **S. Waters-Metenier:** None. **J. Diedrichsen:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.06/GGG3

Topic: D.17. Voluntary Movements

Support: PRESTO of JST

Grant-in-Aid for Scientific Research (C) from MEXT

Title: Compensatory changes in neuronal firing in the perilesional motor cortex: A single unit recording study in the macaque monkey

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Abstract: We previously reported that motor training after lesioning of the primary motor cortex (M1) induces recovery of dexterous hand movements in macaque monkeys (Murata et al., 2008; J Neurophysiol, 99, 773-786). Our recent brain imaging and reversible pharmacological inactivation studies suggest that the recovery involves both the ipsilesional ventral premotor cortex and perilesional M1 (Murata et al., 2012; Soc Neurosci Abstract, 185.04). In the present study, we performed single unit recording in the same M1-lesion model to investigate

compensatory changes of the perilesional motor cortex after M1 lesion at a single neuron level. Single unit activity was recorded during a small-object retrieval task in which the monkey reaches for, grasps and retrieves an object, and moves the hand toward the mouth to eat the food morsel. The movement representation of the recording sites was then confirmed by conventional intracortical microstimulation (ICMS, 1-100 μA, 200 μs in duration, monophasic cathodal pulses at 333 Hz). Before lesion, response property of M1 neurons largely corresponds to the movements of the body part represented in the ICMS-derived movement representation map; e.g., neurons in the digit representation have a peak activity during grasping/retrieving while those in the proximal representation a peak activity during reaching. After the lesion in the hand digit area of M1 by ibotenic acid injection, monkeys could not perform the retrieval task because of the hand paralysis including a complete loss of digit movement. Functional recovery of hand movements was observed, and the number of successful trials in the small-object retrieval task progressively increased during the first month after the lesion. Unexpectedly, the ICMS threshold for inducing the body movements increased in perilesional area after recovery, and there was large area in which ICMS up to 100 μA elicited no movement. Nevertheless, single unit recording after recovery indicated that the percentage of neurons in the perilesional area that have a peak activity during grasping/retrieving was higher than that in the same area before lesion. The present results suggest that the compensatory changes in neuronal firing occurs in the perilesional area, and the changes may underlie functional recovery of hand movements after lesion in the hand digit area of M1.

Disclosures: N. Higo: None. **N. Kunori:** None. **I. Takashima:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.07/GGG4

Topic: D.17. Voluntary Movements

Title: Enlarged striatal brain volume in individual top athletes

Authors: *M. TAUBERT[1], U. WENZEL[2], B. DRAGANSKI[3], S. KIEBEL[1], P. RAGERT[1], J. KRUG[2], A. VILLRINGER[1];
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Abstract: Behavioral studies have shown that many characteristics of elite performers, once believed to reflect innate talent, are actually the result of intense practice extended for a minimum of 10 years (Ericsson et al., 1993). Interestingly, already a short period of motor practice induces structural changes in the brain (Draganski et al., 2004). Since highly automatic movements are coded in the cortico-striatal system (Doyon & Ungerleider, 2002), we assessed morphological differences in striatal regions between top athletes and normal controls.
We employed a case-control study design with 92 male control subjects and two male track and field athletes at an international level. The first athlete was a javelin thrower with a personal best mark > 80 m. He/She is a former national champion in javelin throw and participated in international tournaments including Olympic Games. The second athlete is a long jumper with a personal best mark of > 8 m. He/She is a former national vice-champion in long jump and participated in international tournaments including a World cup.
T1-weighted MRI data from all subjects were acquired on a 3T scanner. MRI data from the javelin thrower was acquired two times separated by two years (Olympic Games in between). T1-data was used to investigate sub-cortical grey matter volume (GMV). For each athlete, we used 46 control subjects from the local database (max. number of matched control subjects from the database). Control subjects were carefully matched for gender, age (± 2 years) and education (German A-Level). Control subjects participating in competitive sports or playing a musical instrument were not included in the study. T1-weighted images were preprocessed using VBM 8 (DARTEL). We compared each athlete's GMV map with those of 46 control subjects using a two-sample t-test (Mühlau et al., 2009). FWE correction was used for multiple comparison correction at $p<.05$ (voxel-level).
Compared to 46 control subjects, larger GMV in the javelin thrower was seen in left and right ventral striatum. No reductions were observed. Striatal increases were also seen two years after the first MRI scan. Furthermore, in the long jumper, larger GMV was observed in the right striatum. No reductions could be identified.
Although limited by the cross-sectional design of our study, we interpret the differences as effect of training on brain structure. The predominant change within the ventral striatum might be related to the mental effort of long-term deliberate practice (Erickson et al., 1993). To the best of our knowledge, this is the first study describing prominent features in brain structure of internationally known track and field athletes at an individual level.

Disclosures: M. Taubert: None. **U. Wenzel:** None. **B. Draganski:** None. **S. Kiebel:** None. **P. Ragert:** None. **J. Krug:** None. **A. Villringer:** None.

Poster

566. Voluntary Motor Plasticity

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.08/GGG5

Topic: D.17. Voluntary Movements

Title: Dose-response effects of anodal transcranial direct current stimulation (tDCS) on cortical excitability in rats

Authors: H. J. HULSHOF, J. REIS, A.-K. GELLNER, C. WEILLER, *B. FRITSCH;
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Abstract: Anodal transcranial direct-current stimulation (tDCS) has been shown to modulate cortical excitability and improve motor learning. By convention human tDCS studies are generally conducted with low stimulation intensities of approximately 0.4-0.8 A/m² to ensure safe levels. Since higher stimulation levels might further improve plasticity, i.e. learning, dose-response studies on functional measures of cortical excitability and possible adverse effects of tDCS are highly desirable. In the current study we aimed to assess the dose-dependent effects of anodal tDCS on cortical excitability in an in vivo rat model of tDCS by studying the effects on sensory evoked potentials (SEP; low stimulation intensities: 0.8 and 8.0 A/m2) and EEG architecture (higher intensities: 8.0 - 47.8 A/m2).
For this purpose, adult male Sprague-Dawley rats were equipped with electrodes for tDCS stimulation above the left primary motor cortex and the chest region. Furthermore, EEG electrodes were surgically placed above the somatosensory cortices and the cerebellum (grounding electrode). In separate sessions, rats were exposed to anodal tDCS of different intensities for a period of 20 min. EEG and SEPs were continuously recorded prior to, during and after tDCS, in freely moving or anaesthetized animals, respectively.
Anodal tDCS at low stimulation intensities (0.8 and 8.0 A/m2) had no significant effect on SEPs or EEG architecture. Mild EEG alterations, with more spiky waves and higher amplitudes, were first observed at a stimulation intensity of 15.9 A/m². This change in EEG patterns was reflected in total EEG power, which showed a significant, short-term increase during stimulation, attributed to changes in the delta, theta and alpha frequency bands. This change in EEG power also appeared to be time-dependent. At an intensity of 15.9 A/m2 a longer stimulation period was required to significantly increase EEG power when compared to stimulation intensities of 31.8 and 47.8 A/m2. Furthermore, whereas in the 31.8 A/m2 group EEG power stayed increased during the second half of the stimulation period, EEG power already decreased during the last 10 minutes in the 47.8 A/m2 group.
In conclusion, the current data show that anodal tDCS affects cortical excitability in a dose-dependent manner, visible as EEG changes but not as SEP changes (only low stimulation intensities were applied in the SEP experiment). While higher simulation intensities showed an increase in cortical excitability, thereby probably improving plasticity, the decrease in EEG power in the highest intensity group might suggest a negative influence of this intensity on cortical excitability.

Disclosures: H.J. Hulshof: None. **B. Fritsch:** None. **J. Reis:** None. **A. Gellner:** None. **C. Weiller:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.09/GGG6

Topic: D.17. Voluntary Movements

Support: NSERC

Title: Cortical adaptations within and between the primary motor cortices after bimanual training and theta burst stimulation to the left dorsal premotor cortex

Authors: *J. L. NEVA[1], M. VESIA[2], A. M. SINGH[1], R. J. IBEY[1], W. R. STAINES[1,3]; [1]Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada; [2]Div. of Neurol. and Krembil Neurosci. Ctr., Toronto Western Res. Inst., Toronto, ON, Canada; [3]Heart and Stroke Fndn. Ctr. for Stroke Recovery, Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstract: Bimanual visuomotor training (BMT) augments the excitability of cortical areas involved in motor preparation [1] and execution [2]. These changes in excitability are characterized by an increase in the cortical territory of the trained muscles within the left primary motor cortex (lM1) [2]. The specific neural mechanisms underlying this effect are unclear, but may involve transcallosal connections between homologous M1 representations and/or connections from the left dorsal premotor cortex (lPMd). Intermittent theta burst stimulation (iTBS) enhances motor evoked potentials (MEPs) and short-interval intracortical inhibition (SICI), with no effect on intracortical facilitation (ICF), when applied to the stimulated M1 [3]. Further, cTBS applied to lPMd suppresses MEPs, with no effect on SICI or ICF in lM1 [4]. Recently it has been found that MEP amplitude of the entire ECR representation in lM1 is enhanced due to iTBS to lPMd. This study investigates the possible intracortical and interhemispheric modulations of the extensor carpi radials (ECR) in M1 bilaterally due to: 1) BMT, 2) iTBS to lPMd, and 3) the combination of these interventions. This study tests three related hypotheses: 1) BMT will enhance excitability within and between M1 bilaterally, 2) iTBS to lPMd will primarily enhance lM1 excitability, and 3) the combination of these interventions will cause a greater enhancement of bilateral M1 cortical excitability. This study will quantify MEPs, SICI, ICF, long-interval intracortical inhibition (LICI), cortical silent period (cSP), and interhemispheric inhibition (IHI) for the ECR in M1 bilaterally. Preliminary results indicate that MEPs are facilitated in both hemispheres, with a decrease in ICF and IHI in both hemispheres

due to BMT. These results demonstrate the possible neural mechanisms that may underlie the early indications of rapid functional plasticity associated with BMT, which may be related to a decrease of inhibition between the M1 homologous representations and modulation of intracortical excitability locally within M1. The intracortical and interhemispheric effects of iTBS to lPMd and the combined effects of iTBS to lPMd with BMT are currently being tested.

1. Smith & Staines 2006. Brain Research, 1:165-174.
2. Neva et al. 2012. Neuroscience Letters, 514:147-151.
3. Huang et al. 2005. Neuron, 45:201-206.
4. Huang et al. 2009. Clinical Neurophysiology, 120:796-801.

Disclosures: J.L. Neva: None. **M. Vesia:** None. **A.M. Singh:** None. **R.J. Ibey:** None. **W.R. Staines:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.10/GGG7

Topic: D.17. Voluntary Movements

Title: Representation of motor speed in the cerebellar anterior lobe

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Abstract: In athletics, motor performance is determined by different abilities such as technique, endurance, strength and speed. Based on animal studies the latter is thought to be encoded in the basal ganglia, sensorimotor cortex and the cerebellum (Turner et al., 2003). Especially the cerebellum seems to play a major role for speed performance since correlations between discharge rate of Purkinje cells and movement velocity have been observed (Hewitt et al., 2011). The question arises whether there is a unique structural feature in the human brain, which allows power athletes to perform a simple movement significantly faster than endurance athletes.
We acquired functional and structural brain imaging data from 32 track-and-field athletes. 16 of whom were “power athletes” requiring high speed foot movements (sprinters, jumpers, throwers) and 16 were endurance athletes (distance runners). We tested maximum movement velocity of plantarflexion (PF-Vmax) by 3D-movement-analysis. fMRI scans were used to identify speed specific regions of interest during fast and slow foot movements and T1-weighted scans to assess structural grey matter volume (voxel based morphometry).

fMRI indicates that fast plantar flexions are accompanied by increased activity in cerebellar anterior lobe ($p < 0.05$; FWE corr.). The same region shows increased grey matter volume for the power athletes compared to their endurance counterparts ($p = 0.002$; FWE corr.). Behaviorally, a significant difference between the two groups of athletes was noted in the maximum speed of plantar flexion (2.03 ± 0.34 m/s power and 1.79 ± 0.20 m/s endurance athletes; $p = 0.007$). Furthermore there was a significant correlation between the regional cerebellar grey matter volume and PF-Vmax. Our results suggest that motor speed of plantar flexion is represented in the anterior lobe of cerebellum and that there are speed-dependent morphological differences between power and endurance athletes. Grey matter differences may be a structural manifestation of the continuously higher demand to generate high discharge rates of Purkinje cells and explosive muscle contractions.

References:

Hewitt, A.L., Popa, L.S., Pasalar, S., Hendrix, C.M. & Ebner, T.J. (2011). Representation of limb kinematics in purkinje cell simple spike discharge is conserved across multiple tasks. J.Neurophysiol., 106, 2232-2247.

Turner, R. S., Desmurget, M., Grethe, J., Crutcher, M. D., Grafton, S. T. (2003). Motor Subcircuits Mediating the Control of Movement Extent and Speed. J. Neurophysiol. 90: 3958-3966.

Disclosures: U. Wenzel: None. **M. Taubert:** None. **P. Ragert:** None. **A. Villringer:** None. **J. Krug:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.11/GGG8

Topic: D.17. Voluntary Movements

Support: PRESTO, Japan Science and Technology Agency

Robertson Neuroscience Investigator, The New York Stem Cell Foundation

Packard Fellowship, David & Lucile Packard Foundation

Pew Scholars Program, The Pew Charitable Trusts

Sloan Research Fellowship, Alfred P. Sloan Foundation

Title: Motor cortex ensemble dynamics imaged during motor learning

Authors: *A. PETERS, T. KOMIYAMA;
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Abstract: The motor cortex is capable of substantial plasticity during learning to flexibly control movements. Previous studies have examined short-term plasticity on the neuronal scale and long-term plasticity on a global scale. Our study is designed to investigate long-term changes within large populations of layer 2/3 neurons accompanying the learning of a skilled motor task. The genetically encoded calcium indicator GCaMP 5G was injected into transgenic mice expressing a red fluorescent protein in all inhibitory neurons, allowing for simultaneous activity recording and classification of excitatory and inhibitory neurons. Mice were fitted with a chronic cranial window over the forelimb area of the motor cortex to allow for weeks of stable imaging. Following recovery, the animals were simultaneously imaged and trained in a cued forelimb task for two weeks. An auditory cue marked a period where the mice could press a piezoelectric lever under the forelimb contralateral to the imaged cortex for a water reward. Animals' performance of the task improved during the first week and stabilized thereafter. Activity of motor cortex neurons was highly correlated to movement. The activity of inhibitory neurons matched the activity of excitatory neurons on a movement-by-movement basis. Over the course of learning, the populations of excitatory neurons associated with movement changed over time, while those of inhibitory neurons remained relatively stable. Within the early learning phase of the first few days, the population of movement-related excitatory neurons significantly increased. Following this initial increase, the number of movement-related neurons decreased to a stable fraction near the end of the training sessions. This population change consisted largely of transient newly-recruited neurons during the early sessions, followed by the pruning of the movement-related pool. In addition to these changes in the identity of movement-related neurons, the temporal structure of ensemble activity became progressively more stable during learning. These results suggest that the stabilization of spatiotemporal activity patterns of motor cortex ensembles is a major component of motor learning.

Disclosures: A. Peters: None. **T. Komiyama:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.12/GGG9

Topic: D.17. Voluntary Movements

Support: R01 HD065438

NSF Graduate Research Fellowship

Title: The effects of skill learning versus skill repetition on cortical organization and behavioral output: A computational model

Authors: *A. S. BAINS, N. SCHWEIGHOFER;
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Abstract: Motor cortex area M1 in rodents and primates consists of a somatotopic map that represents the ability of neighborhoods of neurons, when stimulated, to elicit responses from various body parts. In the example of primate reaching and grasping, specific neighborhoods driving the hand, wrist, and more proximal limb segments have been identified (Nudo et al 1996). It is widely accepted that these neighborhoods in the map can change size and shape in response to motor skill repetition, even in adults. To elicit such changes, the repetition must lead to learning a new skill, likely through Hebbian plasticity-like LTP (Rioult-Pedotti et al 2000), and not simply be repetition of an already-learned skill. For instance, monkeys repeatedly retrieving a food pellet from a small well, requiring learning of a dexterous action, exhibited map changes, while monkeys repeatedly retrieving food from a large well, requiring no new skill learning, did not exhibit changes (Plautz et al 2000, Dancause and Nudo 2011).
Other investigations have conversely suggested that learned-skill repetition may lead to further learning or adaptation. This was shown in a study in which participants repeatedly reached in directions close to a fixed target without exact error feedback, but with a "bonus score" feedback dependent in part on how close they moved to the target. This skill was already well-learned; however, it was found that when the participants reached towards targets in different directions from the original target, their movements were biased towards the original (Verstynen and Sabes 2011). The authors explained this finding using a neural network model with Hebbian learning that transformed target directions to reach directions. The Hebbian learning expanded the representations in the network of recently-repeated target directions on a fast time scale.
Here we attempt to reconcile these observations- that repetition of learned skills does not alter the M1 somatotopic map significantly, while biases driven by such repetition could be explained by Hebbian learning-dependent increases in those skills' representations. We propose a neural network model in which reward-gated Hebbian learning further strengthens defined synaptic pathways when a learned skill is repeated- in this case reaching to a target using a feedback-controlled model arm. This causes bias towards that skill's execution but little change in the motor output map of the network. However, a combination of reward-gated Hebbian learning and exploration of new network activity patterns acts to more fundamentally change the existing synaptic pathways only when a new skill is learned, leading to larger changes in the output map.

Disclosures: A.S. Bains: None. **N. Schweighofer:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.13/GGG10

Topic: D.17. Voluntary Movements

Support: Neurological Foundation of New Zealand Grant 1128-PG

Title: Individualising theta burst stimulation to optimise motor cortex plasticity

Authors: *P. BROWNJOHN, J. N. J. REYNOLDS, N. MATHESON, J. FOX, J. B. H. SHEMMELL;
Univ. of Otago, Dunedin, New Zealand

Abstract: Theta burst stimulation (TBS), consisting of trains of high frequency 50Hz stimulation repeated in a 5Hz theta pattern, has been shown to modulate excitability of the human motor cortex and corticospinal tract when applied using transcranial magnetic stimulation. Intermittent TBS (iTBS) and continuous TBS (cTBS) induce opposing facilitation and inhibition of the motor cortex, through processes thought to be linked to long term potentiation and long term depression, respectively. While a theta frequency of 5Hz is commonly employed in TBS applied to the human motor cortex, this frequency derives from the firing rates of hippocampal neurons in rats, and fails to consider the firing rate of human cortical neurons or subject-to-subject variations in cortical rhythms. To address this we compared the effects of standard 5Hz iTBS and cTBS with individualised TBS paradigms on corticospinal excitability and inhibitory cortical circuits, with the 5Hz theta frequency component of the TBS intervention replaced by the dominant cortical frequency for each individual. Ten healthy volunteers were treated with standard and individualised iTBS (iTBS 5; iTBS I) and cTBS (cTBS 5; cTBS I), and sham TBS, in a randomised order. The effects of these interventions on motor evoked potential (MEP) amplitude, a measure of corticospinal excitability, and short interval cortical inhibition (SICI) and cortical silent period (CSP) duration, measures of inhibitory cortical circuits, were assessed. Both iTBS 5 and iTBS I interventions significantly facilitated MEP amplitude to a similar extent when compared with sham treatment. Unexpectedly, neither cTBS 5 nor cTBS I intervention depressed MEP amplitude when compared with sham, and moreover, cTBS 5 had a mild facilitatory effect. None of the TBS protocols had any significant effects on inhibitory pathways when compared with sham, as assessed by SICI and CSP measurement. Hypothesising that an interaction between cTBS and voluntary muscle activity required for CSP measurements was nullifying the expected cTBS-induced inhibition of corticospinal excitability, we performed an additional session of cTBS 5 in 5 of the 10 subjects, in which CSP measurement was omitted. Under these conditions, cTBS 5 again increased MEP amplitude. We report that individualising facilitatory iTBS to a subject's underlying cortical rhythm had no effect on the magnitude of

corticospinal facilitation induced. Furthermore, in our hands, reportedly inhibitory cTBS protocols produced mild facilitation of corticospinal excitability.

Disclosures: P. Brownjohn: None. **J.N.J. Reynolds:** None. **J.B.H. Shemmell:** None. **N. Matheson:** None. **J. Fox:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.14/GGG11

Topic: D.17. Voluntary Movements

Support: MEXT Grant-in-Aid for Young Scientists (A)

KAKEN C23500617

Title: Timing- and activity- dependent plasticity of indirect cortico-motoneuronal pathways in humans

Authors: *T. NAKAJIMA[1], T. KOMIYAMA[2], H. OHTSUKA[2], S. SUZUKI[2], G. FUTATSUBASHI[2], Y. OHKI[1];
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Abstract: We previously reported that repetitive combined stimulation (RCS) of pyramidal tract and peripheral nerve induces the long-term potentiation (LTP) of indirect cortico-motoneuronal (C-M) excitations which would be mediated via. cervical propriospinal neurons (PNs) in humans. However, underlying mechanisms of the effects are still unclear. Therefore, we further examined characteristics of the modulation of C-M excitations after RCS.
Healthy volunteers, who all gave written informed consent, participated in the experiments. Electromyograms were recorded from the right biceps brachii (BB) and first dorsal interosseous muscles. RCS intervention (0.2 Hz) was performed for 10 min, during which transcranial magnetic stimulation (TMS) to the contralateral motor cortex was delivered in conjunction with the right ulnar nerve stimulation, with or without weak contraction of BB. Interstimulus interval (ISI) for the combined stimulation was set at -10, 2 or 10 ms (negative value: TMS ahead), though simultaneous inputs by both stimuli on PNs was thought to be obtained at an ISI ~7.0 ms. Stimulus strengths were determined to observe the maximum spatial facilitation effect in BB before the intervention, by converging inputs on PNs with a ISI of 10 ms. To observe LTP in C-M excitations in BB, motor evoked potentials (MEPs)

were evoked by TMS at ~0-70 min after the intervention. As reported previously, MEP amplitudes were enhanced after RCS (ISI 10 ms) with BB contraction, which lasted for 20-60 min. However, long-term depression (LTD), instead of LTP, was observed after the RCS without contraction. Furthermore, even with BB contraction, LTP (ISI 10 ms) and LTD (ISI -10 and 2 ms) could be observed under different ISIs after RCS. These findings suggest that plastic changes in the indirect C-M pathways could be induced by RCS, which were strongly timing- and activity- dependent. These changes were resembled the characteristics of synaptic plasticity in CNS. The timing to observe LTP indicates that the changes took place in synapses at the pyramidal tract on cervical PNs.

Disclosures: T. Nakajima: None. **T. Komiyama:** None. **H. Ohtsuka:** None. **S. Suzuki:** None. **G. Futatsubashi:** None. **Y. Ohki:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.15/GGG12

Topic: D.17. Voluntary Movements

Support: Grants-in-Aid for Young Scientists (No. 19680020 to M.M. and 22680031 to K.K.) from MEXT, Japan

Scientific Research on Innovative Areas “Mesoscopic Neurocircuitry” (No. 22115005 to M.M. and 23115504 to K.K.) from MEXT, Japan

Scientific Research on Innovative Areas “Neural Creativity for Communication” (No. 22120520 to Y.I.) from MEXT, Japan

Scientific Research (No. 23300148 to M.M.) from MEXT, Japan

JSPS Fellows (No. 22136 to R.H.)

Research Activity Start-up (no. 23800071 to Y.M.) from MEXT, Japan

Title: Layer-specific dynamics of cortical ensembles and single neurons during motor learning

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Sci. Institute, Tamagawa Univ., Machida, Japan; [5]Dept. of Mol. Therapy, Natl. Inst. of Neuroscience, NCNP, Tokyo, Japan

Abstract: Motor performance improves with repetitive training, and it has been proposed that this is mediated by functional and structural reorganization of the motor cortex. However, it is poorly understood how different cortical neuronal activities in layers 2/3 and 5a are reorganized during learning of a motor task. In this study, we used mice performing a self-initiated lever-pull task (Hira et al., 2013) to show that the neuronal activities that predict the lever trajectory are dynamically reorganized in layer-specific manners during long-term training. Two-photon calcium imaging in layers 2/3 and 5a of the primary motor area was conducted while mice practiced the motor task for 14 consecutive days. In layer 2/3, **no overall change in the accuracy of neuronal ensemble prediction of lever trajectory was detected** during sessions. At the single-neuron level, neurons that increased the prediction accuracy were counterbalanced by those that decreased it, and a subset of neurons maintained high prediction ability. In layer 5a, the ensemble prediction steadily improved and one-third of neurons slowly improved their prediction ability and strongly contributed to the ensemble prediction by the late stage of learning. Our results provide a novel microcircuit model that consists of distinct intermediate layers upstream of layer 5b with the motor output activity. The balanced dynamic network in layer 2/3 may represent coordination of signals from other areas and combination of motor primitives, whereas the downstream layer 5a may constitute the evolving dynamic network that represents a well-learned movement. Layer 5a in the primary motor cortex may be a major site for long-term motor memory.
Reference: Hira et al. Spatiotemporal dynamics of functional clusters of neurons in the mouse motor cortex during a voluntary movement. J. Neurosci. 33, 1377-1390, 2013.

Disclosures: M. Matsuzaki: None. **Y. Masamizu:** None. **Y.R. Tanaka:** None. **Y.H. Tanaka:** None. **R. Hira:** None. **F. Ohkubo:** None. **K. Kitamura:** None. **Y. Isomura:** None. **T. Okada:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.16/GGG13

Topic: D.17. Voluntary Movements

Support: NSERC

Title: Modulation of paired associative stimulation-induced plasticity following aerobic exercise

Authors: *A. M. SINGH[1], J. L. NEVA[1], W. R. STAINES[1,2];
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Abstract: The effects of aerobic exercise on primary motor cortical (M1) excitability are unclear. Given the beneficial effects of acute exercise on cognition, there is strong interest in the ability of exercise to promote cortical plasticity, particularly when paired with skilled tasks. Research from our lab indicates that aerobic exercise can facilitate excitability changes associated with motor learning; however, the outcomes are variable. Thus, in this study, we employed paired associative stimulation (PAS), an established technique shown to induce short-term plasticity in the motor cortex, in order to remove variations in performance and examine the effect of exercise on the induction of plasticity in M1. In addition, we sought to correlate changes induced by PAS with modulations of short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF). It was hypothesized that a single bout of moderate intensity aerobic exercise would lower intracortical inhibition and result in a greater modulation of M1 plasticity associated with PAS. Following identification of the motor hotspot of the abductor pollicis brevis (APB) muscle, transcranial magnetic stimulation (TMS) was applied over the left hemisphere APB representation at varying intensities in order to generate an input-output curve. Subsequently, paired-pulse TMS was used to probe SICI, LICI, and ICF at the central site. All subjects underwent two sessions at least one week apart, consisting of either PAS alone or PAS preceded by exercise. The PAS protocol comprised 180 pairs of median nerve stimulation (at 300% of perceptual threshold) at the right wrist followed by a single suprathreshold TMS pulse over the right APB hotspot, delivered at 1 Hz with a 25 ms inter-stimulus interval. In the control condition, excitability was assessed at the hotspot before, immediately after, and 30 minutes after PAS. In the exercise condition, participants performed 20 minutes of moderate-intensity stationary biking at 65-70% of their age-predicted maximum heart rate prior to undergoing PAS, with identical pre- and post-measurements. Changes were quantified in the amplitudes of motor-evoked potentials (MEPs) in the input-output curves, and the degree of SICI, LICI and ICF at each timepoint. Preliminary results from input-output curves indicate that that the combination of exercise and PAS can facilitate plasticity to a greater degree than PAS alone, and that such changes are associated with greater modulations in intracortical inhibition. The findings presented have implications for both learning-related and rehabilitation-focused plasticity.

Disclosures: A.M. Singh: None. **J.L. Neva:** None. **W.R. Staines:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.17/GGG14

Topic: D.17. Voluntary Movements

Support: NSERC

CIHR

AIHS

Title: CB1 receptor signaling affects motor map expression in rodents

Authors: *K. A. SCULLION, A. T. HUSSIN, M. N. HILL, Q. J. PITTMAN, G. C. TESKEY; Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Motor maps are the topographical representation of movements in the motor cortex and are derived by stimulating pools of layer 5 pyramidal neurons that give rise to the cortical spinal tract. Previously it has been shown that motor map expression can be altered by experience, as well as by the quantity and type of neurotransmitters present in the neocortex. The balance between cortical excitation and inhibition determines motor map expression. Previous research has demonstrated that an increase in cortical inhibition results in an increase in movement thresholds and smaller forelimb motor maps.

Endocannabinoids are retrograde neurotransmitters that regulate neurotransmitter release at many different types of synapses and may therefore modulate cortical excitability. Endocannabinoids and the cannabinoid (CB) receptors are found in layer 5 of the neocortex, the source of the cortical spinal tract.

We examined the role of CB1 receptors on the expression of cortical forelimb motor maps and on activation of layer 5 pyramidal neurons in motor cortex slices. We tested the hypothesis that activation of CB1 receptors in the motor cortex causes an increase in cortical inhibition.

We made the following 3 predictions: 1) CB1 receptor knockout mice will have lower forelimb movement thresholds than wildtype mice, 2) Arachidonoyl 2-chloroethylamide (ACEA), a CB1 receptor agonist, will increase forelimb movement thresholds while 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide (AM 251), a CB1 receptor antagonist, will decrease forelimb movement thresholds, 3) ACEA will increase, while AM 251 will decrease the activation threshold of pyramidal neurons in layer 5 of the motor cortex in response to intracortical microstimulation (ICMS).

To test the first and second predictions, adult mice and rats underwent high-resolution ICMS to map forelimb (digit, wrist, elbow, shoulder) movement representation areas. To test the third

prediction, whole cell voltage and current recordings were carried out in motor cortex slices. Afferents to these layer 5 pyramidal neurons were stimulated using the same in vivo ICMS parameters ("slice ICMS") to study the effects of CB1 receptor agonists and antagonists on single-cell electrophysiological properties and stimulation responses.
Our results indicate that CB1 receptor activation reduced excitatory responses in layer 5 pyramidal cells while inactivation had either the opposite or no effect. Taken together our results indicate that endocannabinoid CB1 receptors play an inhibitory role on motor map expression.

Disclosures: K.A. Scullion: None. **A.T. Hussin:** None. **M.N. Hill:** None. **Q.J. Pittman:** None. **G.C. Teskey:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.18/GGG15

Topic: D.17. Voluntary Movements

Support: BFNT

Title: Transient directed microglia motility and activation by direct current stimulation (DCS): Simultaneous DCS and two photon *In vivo* imaging

Authors: *A.-K. GELLNER, C. WEILLER, J. REIS, B. FRITSCH;
Neurol., Univ. of Freiburg/ Neurocenter, Freiburg, Germany

Abstract: Introduction: Direct current stimulation (DCS) is a widely used experimental tool for non-invasive modulation of cortical excitability and learning. Some light has been shed on neuronal effects of DCS. However, the vast majority of brain cells, glia, has been shown to participate in neuroplasticity and to be electrically active but mechanistically neglected so far. Fluorescent neocortical cells can be monitored through a cranial window by 2-photon in vivo microscopy (2PM). To monitor online effects of DCS on microglia we established combined DCS and 2PM in transgenic mice with fluorescent microglia (heterozygous Cx3Cr1(+/-)-GFP mice). Mechanistically we also utilized homozygous Cx3Cr1(-/-)-GFP mice, which bear a reduced capability of microglial activation.
Methods: Adult transgenic mice were equipped with a combined chronic cranial window -DCS electrode preparation over the left primary motor and somatosensory area. After 14-28 days imaging of microglia in layer II/III by 2PM started. Pre-intervention day: 1 hr of baseline imaging; intervention day: 20 min of pre-, during- and post-DCS imaging each; post-intervention day: 1 hr of imaging. Stimulation polarity was assigned randomly and performed in a cross-over

design with a minimum of 5 days in-between.
Changes in microglial motility including the direction in relation to the stimulation electrode and morphology were analyzed by ImageJ.
Results: DCS electrodes can be successfully implemented in a chronic cranial window preparation and enable longitudinal 2PM including image acquisition during DCS. We found a significant increase in microglia motility during anodal and cathodal DCS. Directed migration occurred towards the cathode and away from the anode. Motility was normalized to baseline values 1 day after DCS. Analysis of cell morphology revealed remarkable signs of activation within the 40 min of DCS and post-DCS. This effect was more pronounced in slightly pre-activated microglia compared to those in resting state. All of these features were inhibited in Cx3Cr1(-/-) mice.
Conclusion: For the first time we demonstrate feasibility of combined DCS and 2PM. Our study reveals the capability of DCS to increase microglial motility with polarity dependent features and to induce activation. The inhibition of these effects in Cx3Cr1(-/-) genotype suggests a mechanistic role of fractalkine-signalling in DCS induced microglial activation. Further studies elucidating the potential role of microglia as contributor to DCS mediated neuroplasticity are needed.

Disclosures: A. Gellner: None. **C. Weiller:** None. **J. Reis:** None. **B. Fritsch:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.19/GGG16

Topic: D.17. Voluntary Movements

Support: CNPq

FAPERJ

REDE D'Or São Luiz

Title: Neurofeedback training over the premotor cortex increases the activation of motor related areas

Authors: *E. C. RODRIGUES[1,2], T. F. MARINS[3,4], A. ENGEL[3], I. BRAMATI[3], R. BASILIO[3], R. LENT[4], J. MOLL[3], F. TOVAR-MOLL[3,4];
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Abstract: Real-time functional magnetic resonance imaging (fMRI) neurofeedback (NFB) allows individuals to voluntarily achieve control and modulate different areas of their own brains. The NFB can be associated with motor imagery, that is the mental rehearsal of a motor act, without overt body movement. This combination can potentially modulate the activation of cortical areas that are typically involved in motor planning and execution. The objective of this work was to investigate if the activity of the left premotor cortex (PM) could be modulated by motor imagery of the right hand finger tapping with NFB training. The PM was selected as the region of interest because it is an important motor related area that may play a key role in rehabilitation after brain injury. Twenty right-handed healthy volunteers were included in the study and divided into 2 groups, NFB (n=10) and controls (n=10). The experimental design was composed by a pre-training session and a 3T MRI acquisition, including high-resolution spin-echo anatomical sequence (T1-weighted image) and a fMRI protocol (EPI single-shot (TR / TE, 2000 / 22 ms; voxel size: 3,75 x 3,75 x 5 mm3). The fMRI protocol encompassed (i) a motor run (real finger tapping movement) and (ii) three imagery runs. Each imagery run comprised 8 blocks containing 30s motor imagery of the right hand finger tapping, and 20s of a baseline condition (i.e., visual imagery of a scene). All participants viewed through a mirror on top of the head coil a screen presenting a bar graph with dynamically raising or falling content. In the NFB group the changing bar graph was related to the hemodynamic response in the left PM area during motor imagery, providing participants a feedback of their own ongoing brain activation. NFB group was instructed to increase the activation level during training. In the control group, the movement of the bar was random during motor imagery and participants were asked to pay attention to the graph bar. The entire session lasted 45 minutes. An accelerometer was positioned over the thumb to ensure that no overt movement occurred during motor imagery. NFB volunteers were able to increase the left PM cortex activation throughout training session. In addition, direct comparison between the last and first NFB runs showed increased activation in other motor-related brain areas, such as ipsilateral PM cortex, supplementary motor area, basal ganglia and cerebellum for the NFB group, but not for the control one (fixed-effect analysis; $p<0.05$ FWE). In conclusion, NFB training can induce functional plasticity in motor related areas. This finding can be particularly important for clinical application in motor rehabilitation in neurological disorders.

Disclosures: E.C. Rodrigues: None. **T.F. Marins:** None. **A. Engel:** None. **I. Bramati:** None. **R. Basilio:** None. **R. Lent:** None. **J. Moll:** None. **F. Tovar-Moll:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.20/GGG17

Topic: D.17. Voluntary Movements

Support: NIH/NINDS NS078791

Title: Constructing a hodological map for the mouse forelimb muscles

Authors: *E. M. STRAIT, M. BANNECK, Y. ZUO;
MCD, Univ. of California, Santa Cruz, Santa Cruz, CA

Abstract: The motor cortical area representing task-related muscle movements expands during repetitive practice of motor skills. Using in vivo imaging, our previous studies have shown that motor learning promotes formation of new synapses onto layer 5 pyramidal neurons in the motor cortex and these new learning-induced synapses are selectively stabilized with repeated practice of the same task. However, how learning-induced synaptogenesis contributes to circuitry remodeling of the motor system and how projection trajectories of cortical neurons change during the learning process remain unknown. Using multi-colored, retrograde transneuronal pseudorabies viruses (PRVs), we have recently developed a method to identify cortical neurons controlling particular forelimb muscles. We found that it took 3 days for PRVs to reach the cortex after they were injected into forelimb muscles. At this time, only layer 5 cortical neurons were infected in the cortex, with the majority of labeling observed in the contralateral motor cortex. One day later, more layer 5 cortical neurons were infected on both hemispheres and the infection also spread out to other cortical layers. In the cortex, only neurons were infected by the virus, implying faithful transneuronal transport of PRVs. We are currently examining the hodological maps for different muscle groups and determining how such maps change in response to motor skill learning.

Disclosures: E.M. Strait: None. **M. Banneck:** None. **Y. Zuo:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.21/GGG18

Topic: D.17. Voluntary Movements

Support: DFG RE 2740/3-1

Title: Training strategies in the elderly may differentially affect the speed accuracy trade-off during motor skill learning

Authors: M. ELWENSPOEK[1], G. PRICHARD[2], A. SCHÖCHLIN-MARX[1], B. FRITSCH[1], *J. REIS[1];
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Abstract: Acquisition of a new motor skill results in an increased level of motor performance. Motor skill learning on a sequential visual isometric pinch force task results in a shift in the speed-accuracy trade-off function (SAF) in young subjects (Reis et al., 2009). When subjects move through speed-accuracy space, different strategies may be utilized (e.g. mostly increasing speed while maintaining accuracy, or increasing accuracy while maintaining a chosen speed). Hence, similar levels of skill can be reached with different strategies. These strategies have not been investigated so far in elderly subjects. Here we investigated whether a constrained training mode (fast speed versus high accuracy) would result in a specific shift of the speed-accuracy trade-off function relative to an unconstrained mode.
42 healthy elderly subjects practiced the sequential visual isometric pinch task in three different training modes (n=14 per group; as fast as possible versus as accurate as possible versus fast+accurate in unconstrained mode) for 3 consecutive days. We assessed skill by probing the SAF in testing sessions one day before and after the training period.
The SAF probes showed that training resulted in improved accuracy for given speeds encountered during training, but also for speeds never reached during training in all three training modes (generalization). Moreover, our preliminary analysis revealed a more pronounced shift in those speeds assigned to (speed group) or chosen (accuracy group) during training.
These preliminary results suggest that in elderly people: 1) Skill generalizes during training, as untrained speeds can be better performed afterwards. 2) Training strategies may be visible by a change in particular aspects of the SAF. Hence, constrained training strategies may facilitate different components of motor skill learning. These results are of relevance for behavioral and sports science as well as neurorehabilitation.
1. Reis et al. (2009). Proc Natl Acad Sci U S A.106(5):1590-5.

Disclosures: M. Elwenspoek: None. **J. Reis:** None. **A. Schöchlin-Marx:** None. **B. Fritsch:** None. **G. Prichard:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.22/GGG19

Topic: D.17. Voluntary Movements

Support: NIH Grant R01 HD061462

NIDRR H133P100014

Title: Effects of motor cortical stimulation timing on neuroplasticity during planar reaching movement

Authors: *C. L. MASSIE[1], P. NARAYANAN[2], S. S. KANTAK[3], G. F. WITTENBERG[4,2,1]; [1]Physical Therapy and Rehabil. Sci., [2]Neurol., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Moss Rehabil. Res. Inst., Elkins Park, PA; [4]Geriatrics Research, Educ. & Clin. Center, Veterans Affairs Med. Ctr., Baltimore, MD

Abstract: Robotic technology is increasingly used for rehabilitation and the potential exists to enhance use-dependent plasticity with non-invasive motor cortex stimulation specifically timed with the practiced reaching movements. The objective was to determine how the timing of stimulation influenced neuroplasticity associated with robotic reaching. Sixteen participants completed 3 separate sessions with different stimulation timings (pre-movement, EMG triggered, random) during a repetitive reaching intervention using a planar robot with 480 trials of movement. Sub-threshold, single-pulse transcranial magnetic stimulation (TMS) was delivered at: pre-movement - 120-150ms prior to movement onset; EMG triggered - when muscle activity exceeded threshold; or randomly - anytime between visual cue and movement completion. Five assessments included the amplitude and direction of TMS evoked movements with corresponding motor evoked potential (MEP) recordings from the biceps, triceps, anterior and posterior deltoid muscles. Each assessment included 10 averaged trials and MEP data were organized into elbow and shoulder agonist and antagonist groups. Change scores from the initial assessment were calculated as follows: % change in amplitude, absolute change in direction (0-180 degrees), and change in MEP amplitude (normalized to amplitude at movement threshold). Data were analyzed using a general linear model with repeated measures for time and a between subjects effect for stimulation conditions. The evoked movements significantly increased in amplitude following the pre-movement and random conditions, but not following EMG triggered condition ($p < 0.05$). The TMS evoked directions significantly changed following all conditions ($p < 0.05$), and no differences between groups were observed. There was a significant difference between conditions for shoulder and elbow agonist MEPs with a general increase in amplitude following pre-movement and a significant decrease following the EMG triggered condition ($p < 0.05$). These findings demonstrated that stimulation delivered during pre-movement and EMG triggered conditions influenced neuroplasticity with a robotic reaching intervention, yet the mechanism by which these changes occurred appeared to differ. The direction of plasticity may change rapidly and selectively during the planning and production of movement. This dynamic change in plasticity shapes the way in which practice results in motor system changes.

Disclosures: C.L. Massie: None. **P. Narayanan:** None. **S.S. Kantak:** None. **G.F. Wittenberg:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.23/GGG20

Topic: D.17. Voluntary Movements

Support: NHMRC Project Grant

Title: The effects of hand immobilization on motor imagery: A multimodal study

Authors: *L. MARSTALLER[1], P. SOWMAN[1], A. RICH[1], M. WILLIAMS[1], G. SAVAGE[2], H. BURIANOVÁ[1];
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Abstract: Neural plasticity in somatosensory cortex has been investigated extensively in patients with stroke (Hallet, 2001; Pascual-Leone et al., 2005) or phantom limbs (Ramachandran et al., 1996; Karl et al., 2001), and in healthy populations after hand immobilization or motor training (Liepert et al., 1995; Ngomo et al., 2012; Zanette et al., 2004). The aim of this study was to investigate the effects of short-term hand immobilization on motor imagery, and specifically immobilization-related plastic changes in the primary motor cortex (M1). Using a novel motor imagery paradigm (Burianova et al., 2013), we tested 16 healthy, right-handed adults, assessing neural changes in M1 with functional magnetic resonance imaging (fMRI) and related oscillatory changes in the beta frequency band with magnetoencephalography (MEG). We measured resting motor thresholds (RMTs) and motor map areas (MMAs) with transcranial magnetic stimulation (TMS), thus attaining a direct index of the immobilization effect. The results show that after 24 hours of right hand immobilization, RMTs and MMAs increased significantly in the contralateral motor cortex. In addition, fMRI and MEG results show a significant reduction in BOLD activation and beta desynchronization in M1 contralateral to the immobilized hand. The results suggest that short-term hand immobilization has a direct and rapid effect on motor imagery in M1, which can be reliably assessed using multimodal neuroimaging methods.

Disclosures: L. Marstaller: None. **P. Sowman:** None. **A. Rich:** None. **M. Williams:** None. **G. Savage:** None. **H. Burianová:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.24/GGG21

Topic: D.17. Voluntary Movements

Support: Brain Research Trust (6CHB, S.W.M.)

The Wellcome Trust (094874/Z/10/Z, J.D.)

Title: Enhancing effector-independent representations of motor skill with transcranial direct current stimulation (tDCS) to primary motor cortex (M1): Behavioral and neuroimaging effects

Authors: *S. WATERS-METENIER[1,2], T. WIESTLER[1], J. ALLEN[3], M. HUSAIN[1,2,4,5], J. DIEDRICHSEN[1];
[1]Inst. of Cognitive Neurosci., [2]Inst. of Neurol., [3]Sch. of Med., Univ. Col. London, London, United Kingdom; [4]Nuffield Dept. of Clin. Neurosciences, [5]Dept. of Exptl. Psychology, Oxford Univ., Oxford, United Kingdom

Abstract: It has been hypothesized that it is behaviorally advantageous to *increase contralateral* M1 activity and simultaneously *decrease ipsilateral* M1 activity, thereby reducing interhemispheric inhibition of contralateral M1. Indeed, a bihemispheric tDCS montage--with the anode over contralateral and cathode over ipsilateral M1--facilitates motor performance better than unihemispheric tDCS, in which the cathode is above non-motor structures (Vines et al., 2008). To test whether the advantage of bihemispheric tDCS really arises from a suppressive effect on ipsilateral M1, we trained 42 healthy, right-handed subjects for 4 days on a finger sequence task, while applying double-blind sham or bihemispheric tDCS with the anode over contralateral M1 (bi-anodal) or ipsilateral M1 (bi-cathodal). Training was conducted with *either* left or right hand, and tDCS (2mA) was applied during the first 25 min of a 1hr training session. We expected that bi-cathodal tDCS would attenuate learning with respect to sham, as it should not only directly suppress contralateral M1, but also increase ipsilateral M1 activity, further inhibiting contralateral M1. Contrary to this prediction, we found that both bihemispheric tDCS groups achieved ~40% faster movement times than sham recipients, as well as ~20% faster movement times than eight anodal unihemispheric tDCS recipients. This indicates that the additional benefits conferred by bihemispheric relative to unihemispheric tDCS are achieved due to increases in plasticity in *both* hemispheres, rather than suppression of ipsilateral M1. Consistent with this idea, we also found that tDCS-associated learning gains, regardless of montage, near completely generalized to the untrained hand. Further, intermanual generalization was most pronounced in the bi-cathodal group, indicating that this montage particularly influenced effector-independent representations. Finally, we studied the neural effects of tDCS-

coupled training in the same participants using fMRI. We observed that both bihemispheric tDCS groups exhibited ipsilateral increases in % BOLD signal change (as well as contralateral increases), whereas sham recipients showed characteristic ipsilateral deactivation. In conclusion, we propose that, irrespective of polarity, bihemispheric tDCS facilitates dual hemisphere coding of effector-independent representations, whereas unihemispheric tDCS (which is half as effective) enhances such encoding in one hemisphere.
Vines BW, Cerruti C, Schlaug G (2008) Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation. BMC Neurosci 9:103.

Disclosures: S. Waters-Metenier: None. **T. Wiestler:** None. **J. Allen:** None. **M. Husain:** None. **J. Diedrichsen:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.25/GGG22

Topic: D.17. Voluntary Movements

Support: EKFS 2011-119

DFG Fl-379-8/1

FKZ0315673A

01GY1144

01EO0801

ARC FT120100608

Title: Microstructural changes of motor tracts in healthy ageing

Authors: R. LINDENBERG, A. WILLERT, M. MEINZER, *A. FLOEL;
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Abstract: Studies on stroke patients have demonstrated that so-called alternate motor fibers (aMF), which may comprise the cortico-rubro-spinal system, can compensate for damage to the pyramidal tract (PT). While it is well documented that the PT undergoes degeneration with age, no data are available with regard to aMF. Using diffusion tensor imaging (DTI), we examined

microstructural properties of PT and aMF in groups of young and older healthy subjects. We hypothesized that aMF may undergo plastic changes in order to compensate for the previously described age-related degeneration of the PT.
36 healthy older subjects (18 women, mean age 68.0±5.3 years, all right-handed) and 90 healthy young subjects (45 women, mean age 25.1±3.7 years, all right-handed) underwent MRI at 3T. We acquired a DTI sequence (10 b0, 64 b1000, isotropic voxels sized 2.3x2.3x2.3 mm3) in order to extract fractional anisotropy (FA) of the pyramidal tract (PT) and so-called alternate motor fibers (aMF). FSL was used for pre-processing and probabilistic tractography. For both PT and aMF, waypoint masks were used in the posterior limb of the internal capsule and the hand knob of the primary motor cortex. The anterior pons served as seed region to reconstruct the PT, whereas ROIs applied to the posterior pons were used for aMF. We extracted mean FA and mean intensities of the reconstructed tracts in either hemisphere in order to compare values between the two groups. In addition to this tract-specific approach, a slice-by-slice analysis allowed us to investigate potential regional differences between the age groups.
With regard to tract-specific FA, significant between-group differences were found in right PT (lower FA in older adults) and left aMF (higher FA in older adults). The slice-by-slice analysis revealed that FA reductions in the right PT of older compared to younger subjects could be observed throughout the tract, from its origin in the cortex to the brainstem. In contrast, regional differences in left aMF (higher values of older compared to young participants) appeared to be greater in the midbrain and brainstem than in the white matter cranial to the internal capsule.
In conclusion, aMF may not only play a compensatory role in case of PT damage due to lesions such as stroke, but also in physiological ageing. In line with previous studies on stroke patients, our data are suggestive of plastic changes in the vicinity of midbrain and brainstem nuclei of corticofugal motor tracts (e.g., the red nucleus), potentially as a result of age-related PT degeneration. Our study sheds light on reorganization processes of the motor system in ageing, which may be of value for the development of restorative treatments.

Disclosures: R. Lindenberg: None. **A. Floel:** None. **M. Meinzer:** None. **A. Willert:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.26/GGG23

Topic: D.17. Voluntary Movements

Support: VA RR&D F7823S

UF University Professors Scholarship

AHA Grant POST10920002

VA RR&D B6793C

Title: Unilateral activation of the less affected limb to task-failure facilitates the ipsilesional hemisphere post-stroke

Authors: *C. PATTEN[1], E. WHITE[2], N. LODHA[3];
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Abstract: Unilateral movements activate multiple brain regions bilaterally, leading to cross-transfer effects between limbs. Previous investigations suggest the magnitude of bilateral activation scales with contraction force. Here we investigated concurrent neurobehavioral effects of unimanual gripping and to observe acute adaptations in cortical and interhemispheric circuits. We studied 6 persons (63.7±3.8 yrs) with post-stroke hemiparesis (UEFMA 53.5±2.9/66 pts) and 6 age-matched (60±7 yrs) controls.
Participants performed repeated submaximal (30% maximal age-referenced norm) isometric power grip to task-failure. Maximal voluntary isometric grip force (MVIC) was assessed at: baseline and following every 10th submaximal repetition (10 repetitions = 1 block). Single-pulse transcranial magnetic stimulation was used to elicit cortical (cSP) and ipsilateral (iSP) silent periods and motor evoked potentials (MEPs) at: baseline, immediately following, 30 and 60minutes post-task-failure. The more (MA) and less (LA) affected limbs of participants post-stroke were studied in separate sessions one week apart. The non-dominant hand was studied in Controls (C).
At task-failure MVIC grip force was reduced similarly among MA, LA and C hands (range 21.3 - 28.9%, $p > .05$). In contrast, time to task-failure was significantly prolonged (203.5 blocks) and reduced (18 blocks) in LA and MA, respectively, relative to C (120 blocks) hands ($p < .05$).
In activated hemispheres normalized MEParea (i.e., MEParea/Mmax) increased modestly immediately following (Stroke-Ipsilesional (IL), 47.6%), post30 and post60 (C, 74-79%) (p's <.05). In the resting, IL, hemisphere, however, normalized MEParea increased markedly (85.2%, $p < .005$) immediately following LA hand activity to task-failure. This IL hemisphere facilitation was both sustained and extended for up to one hour following activity (post30, 125.6%; post60, 160%, p's <.0002). Concurrently, iSP duration measured in the IL hemisphere, indicating interhemispheric inhibition (IHI) from IL-to-CL, was significantly increased (post, 51%) and sustained for up to one hour (post30, 48%; post60, 64%; p's <.05). Taken together, these results suggest that systematic activation of the LA hand (CL hemisphere) facilitates the IL hemisphere and improves IL-to-CL IHI, reducing inter-hemispheric competition known to occur following stroke. Rather than force magnitude, per se, these effects appear to be mediated by activation to task-failure. Persistence of these neural adaptations for up to one hour may offer a novel therapeutic opportunity for effective rehabilitation of paretic limbs.

Disclosures: C. Patten: None. **E. White:** None. **N. Lodha:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.27/GGG24

Topic: D.17. Voluntary Movements

Support: NIH Grant R01HD073147

Title: Physiological changes in the cerebellum and primary motor cortex during skill learning

Authors: *D. SPAMPINATO[1], A. BASTIAN[2,5], P. CELNIK[3,4];
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Abstract: Learning motor tasks is associated with excitability changes in the cerebellum (CB) and primary motor cortex (M1). Two important types of motor learning include adaptation and skill learning. Adaptation refers to an error-based learning of sensorimotor calibrations, whereas skill learning requires performance improvements with repeated practice via reinforcement mechanisms. Although these two forms of learning have been linked to different neural substrates and physiological changes, it is likely that learning certain skills tasks have an initial component of acquiring a new sensorimotor calibration. Previously, we have shown that CB-M1 connectivity decreases early on when participants experience adaptive motor learning (Jayaram et al., 2011; Schlerf et al., 2012), whereas skill learning results in occlusion of LTP-like plasticity at the end of the practice session (Cantarero et al., 2013). Thus, we predict that if learning a skill involves in its early stages forming a new sensorimotor calibration then CB-M1 connectivity should be reduced early during skill learning but not late, once the calibration has been fully learned. Similar, we would expect that occlusion of LTP-like plasticity should be more prominent late but not early during the skill learning process.
We used a pair-pulsed transcranial magnetic stimulation (TMS) technique to assess the changes in CB-M1 connectivity before, during, and after participants trained on an isometric finger-pinch sequence skill task. In addition, we assessed the magnitude of occlusion early and late during skill training, using anodal transcranial direct current stimulation (TDCS).
We found a reduction in CBI only initially when participants began to learn the skill task ($p<0.05$). However, with further practice, CBI returned to baseline levels despite improvement in performance. Interestingly, we found that CBI changes were proportional to early skill

improvement (R2=0.58, $p<0.05$). In addition, we found that occlusion of LTP-like changes over M1 occurred late but not early during skill learning ($p<0.05$). Importantly, a control group that trained on a randomized form of the task showed no changes in CBI or occlusion of M1. These findings indicate that CB-M1 connectivity changes occur early on during the initial acquisition of a skill, whereas M1 role in skill learning becomes more prominent as the skill is practiced. This suggests that skill learning, which relies in reinforcement practicing mechanisms, also incorporates early on adaptation mechanisms.

Disclosures: D. Spampinato: None. **P. Celnik:** None. **A. Bastian:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.28/GGG25

Topic: D.17. Voluntary Movements

Title: Visual attention load modulates motor cortical plasticity through sensory-motor projections

Authors: *J. L. MIRDAMADI, L. Y. SUZUKI, S. K. MEEHAN;
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Abstract: Repetitive transcranial magnetic stimulation (rTMS) has been shown to induce neural plasticity, providing a potential means of enhancing learning and rehabilitation. However, the efficacy of this technique remains uncertain due its state-dependent nature. Previous work suggests a role for attention in mediating plasticity of the motor cortex. Although, whether attention works directly on the motor cortex or indirectly through altering sensory processing remains uncertain. Here, we addressed this issue by delivering 2-second trains of theta burst stimulation (TBS) over either the motor or sensory cortex under varying attention loads. It was hypothesized that increased attention load would reduce motor cortical excitability changes induced from TBS over the motor cortex, as previously reported. In contrast, we expected that TBS over the sensory cortex would enhance motor cortical excitability, regardless of attention load. 2-second trains of TBS (3 pulses at 50 Hz repeating every 200 msec, 80% AMT) were delivered over either the motor cortical representation of the first dorsal interosseous muscle or the corresponding sensory cortex. During TBS, participants were engaged in a visual detection task that required either high attention or low attention. Motor cortical excitability was quantified using motor evoked potential (MEP) amplitude elicited by single pulses of TMS (120% RMT) before TBS and at 2-second intervals after TBS for 20 seconds. 12 trials were delivered over

each stimulation site (sensory cortex, motor cortex) and for each attention load (low load, high load). As a control, the procedure was repeated without any TBS. Preliminary results (N=8) suggest that 2 seconds of TBS increased MEP amplitude regardless of TBS site. However, the increase in MEP amplitude was greater following sensory TBS compared to motor TBS. For both sites of TBS high attention load reduced MEP amplitude compared to low attention load. Interestingly, MEP amplitude was similar for motor TBS with low attention load and sensory TBS with high attention load. Motor evoked potentials in the absence of TBS demonstrated no change in amplitude regardless of attention load. These results suggest that attention may influence plasticity in the motor cortex, reflected by increased MEP amplitude, indirectly through altering sensory-motor projections. These results may have important implications for maximizing the benefits of TBS in both healthy individuals and potential rehabilitation interventions.

Disclosures: J.L. Mirdamadi: None. **L.Y. Suzuki:** None. **S.K. Meehan:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.29/GGG26

Topic: D.17. Voluntary Movements

Support: NIH R01NS069214-02

Title: Hardware simulation of excessive cortical drive results in increased motoneuron excitability and presents features of spasticity

Authors: *S. NANDYALA, C. M. NIU, T. D. SANGER;
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Abstract: Childhood-onset spasticity can typically take an upwards of 10 years to develop before symptoms present. Disease onset in this time frame suggests there is involvement of a long-term plasticity component in response to the descending motor drive. A biorealistic, hardware simulation of the spinal motor loop over a period of 10 years of simulation time was used to investigate the effects of long-term excessive descending drive on the excitability of alpha and gamma motoneurons. Synaptic plasticity was modeled using the spike-timing dependent plasticity (STDP) and Bienenstock-Cooper-Munro (BCM) learning rules. By using a hardware emulation, we are able to accelerate emulation time to 365x faster than real time, allowing us to investigate these long-term plasticity effects in a reasonable time frame. Using this model, we emulated a variety of stretch reflex responses at a single joint. The emulated

EMG response shows good correspondence with the known velocity-dependent spastic EMG patterns as reported in Jobin et al. 2000. The emulated joint torque response shows good correspondence with known spastic torque responses as reported in Schmit et al. 1999. These results suggest that increased motoneuron excitability in response to excessive cortical drive can explain the causation of some forms of childhood-onset spasticity.

Disclosures: S. Nandyala: None. **C.M. Niu:** None. **T.D. Sanger:** None.

Poster

566. **Voluntary Motor Plasticity**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 566.30/GGG27

Topic: D.17. Voluntary Movements

Support: NSF Grant ECS-0702057

NSF Grant ECS-1002391

Title: Trial-to-trial variability of PM and M1 cortical neurons encodes directional learning control task factors

Authors: *J. SI, H. MAO, T. KETCHUM;
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Abstract: Trial-to-trial variability is recently found informative in neuronal encoding of sensory and motor parameters, though the nature of the variability is elusive. To provide insight on the issue, we studied trial-to-trial firing rate variability of PM and M1neurons as rats learned to associate light cues with appropriate directional control strategies.
A rat would press a center lever to start a trial at his own will. One of five cue lights would appear. The rat could press either a left or right control lever to “move” the cue light to the right or left by one position, respectively. The goal was for the rat to “move” the light to the center for a food reward. By trial and error, rats could reach an accuracy of 80% or above in about 30 sessions on average. Single units in PM and M1 were recorded along with behavioral parameter. In each session/day, trials were grouped as L-L subset (left movement in response to the left side cue) and R-R (right movement to the right side cue). According to trial outcomes, the trials were grouped as P-S (post a previously success trial) and P-E (post a previously error trial). Neuronal spike counts in a 100 ms window sliding at a 20 ms step were computed. The L-L and R-R trials were compared, and direction selectivity (DS) was defined if significant differences were observed (Mann-Whitney *U*-test, $p<0.01$). Similarly, previous outcome selectivity (POS) was

recognized between P-S and P-E trials. A neuron was labeled as DS if direction selectivity was observed in at least 20% of the time from 200 ms to 2000 ms after cue onset, and similarly POS neurons were identified. Among the 678 neurons recorded from all sessions of 8 rats, 24.0% were DS, 26.5% were POS, and 6.6% showed both types of selectivity.
Trial-to-trial neural variability was calculated as spike count variance over mean (Fano factor, FF) in L-L and R-R trials of DS neurons, and P-S and P-E trials of POS neurons, respectively. Decreased FF values were found in L-L, R-R, and P-S trials from 1.76, 1.73, and 2.89 before cue onset to 1.38, 1.52, and 2.47 after (*U*-test, $p<0.01$), respectively. But it was not in P-E trials when FF changed from 2.43 to 2.48 (*U*-test, $p>0.5$). Meanwhile, P-S and P-E trials had higher FF values than L-L and R-R trials.
These results demonstrated that trial-to-trial firing rate variability carried task related information. This variability may suggest different levels of coding demand, where movement parameter coding required relatively more precise rate coding while performance monitoring was coded less precisely on a daily basis.

Disclosures: J. Si: None. **H. Mao:** None. **T. Ketchum:** None.**Poster**

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.02/GGG29

Topic: D.18. Brain-Machine Interface

Support: NIH Director's Pioneer Award

NIH TRO1

DARPA REPAIR

NSF Graduate Research Fellowship

NSF IGERT

Stanford MSTP

Title: Motor cortical activity tracks the position of a brain-machine interface cursor

Authors: *S. D. STAVISKY[1], J. C. KAO[2], P. NUYUJUKIAN[3,4], S. I. RYU[6], K. V. SHENOY[5,2,4];
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Abstract: Brain-machine interfaces (BMIs) continue to move towards restoring movement to individuals with paralysis or limb loss. However, little is known about how the sensorimotor system incorporates BMIs into its motor control strategy. We tested whether a well-described property of arm control - that motor cortical activity is modulated by hand position (Caminiti et al. 1991) - also holds when a task is done with a BMI rather than with the arm. During arm reaches, hand position is linked to neural activity through mechanisms including proprioception, postural tonic output, visual feedback, and efference copy. Moving a cursor via BMI, without arm movements, minimizes the influence of proprioception and postural tone and reveals whether the cursor's position still affects neural activity.

Two macaques were implanted with multielectrode arrays in primary motor (M1) and premotor (PMd) cortex. They performed a 2D task in which a cursor either followed the monkey's fingertip (arm control) or was driven by a BMI that decoded multiunit activity into cursor velocity. During BMI use both arms were restrained. The monkey moved and held the cursor over a target appearing at a random location. We linearly regressed the multiunit firing rate during these hold epochs against hand or cursor position to measure how much neural variance was explained by position. In a representative dataset, during arm control the hand position explained significant variance ($p < .001$, shuffle test) on 118/161 movement-modulated channels ($R^2 \in [0.012, 0.531]$). In BMI control, cursor position explained significant variance on 77 channels ($R^2 \in [0.006, 0.196]$). Since the BMI controlled only cursor velocity, modulation of neural activity due to cursor position suggests that visual feedback and/or an internal model affect the motor system during BMI use. Further work must test if gaze differences contribute to this effect, but we note that our result holds in M1 and that prior studies found weak correlates of free gaze in PMd (Cisek & Kalaska 2002).

We further show that a decoder that accounts for neural modulation expected due to the current cursor position (Gilja et al. 2012) outperforms decoders that instead account for actual hand position (81% longer acquire times) or omit this step (14% longer acquire times). These results are consistent with a feedback controller view of motor cortex in which the BMI cursor is the task-relevant effector whose state is tracked by the sensorimotor system. It provides principled justification for applying an online control perspective to neural-control algorithm development and motivates future studies of how sensory feedback is incorporated into the neural state during BMI use.

Disclosures: S.D. Stavisky: None. **J.C. Kao:** None. **P. Nuyujukian:** None. **S.I. Ryu:** None. **K.V. Shenoy:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.03/GGG30

Topic: D.18. Brain-Machine Interface

Support: Nano Research Facility at Washington University in St. Louis

Title: Microfabrication and finite element modeling of micro and macro sieves for use in targeted peripheral nerve regeneration

Authors: *J. PARDO, E. ZELLMER, D. MORAN;
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Abstract: While human peripheral nerves are known to regenerate following damage, the restoration of normal nerve function following injury has remained, at best, an elusive aim. Withal, it has been suggested and evidenced that modulation of nerve afferent plays a central role in the mechanisms that are important for learning and executing motor tasks. Previous work has shown that peripheral nerve axons regenerate through vias in polyimide sieve guides. Furthermore, electrodes can be integrated onto the polyimide sieve to make a complete multichannel neural interface. A major challenge is the optimal design and fabrication of these sieves to ensure the best possible regeneration. The present study presents the on-going development of a next-generation integrated and flexible sieve interface for use in targeted peripheral nerve regeneration. First, a process technology was developed to fabricate a multilayer sieve device with micron sized features meeting mechanical, electrical and biocompatible requirements. Additionally, the process is shown to be adaptable to multiple geometric designs (micro and macro) which can be tested in terms of regeneration performance. Second, a myelinated mammalian peripheral nerve segment was modeled with a microsieve electrode device, using finite element analysis, to evaluate the spatial selectivity the microsieve electrode, as well as, explore the novel stimulation paradigms that been found useful in increasing selectivity in the previously developed macrosieve electrode.

Disclosures: J. Pardo: None. **E. Zellmer:** None. **D. Moran:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.04/GGG31

Topic: D.18. Brain-Machine Interface

Support: FDA-DARPA IAA 224-10-6006

Title: Assessing neuronal viability near chronically implanted microelectrode arrays using optogenetics

Authors: *G. L. KNAACK[1], E. CIVILLICO[3], K. T. WACHRATHIT[3], S. HUANG[3], J. J. PANCRAZIO[2], C. G. WELLE[3];
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Abstract: Interest in the use of implanted microelectrode arrays for neural prosthetic applications has increased. Implanted neural interfaces are also utilized clinically to relieve the symptoms of neurological disorders such as essential tremor, Parkinson's, and obsessive-compulsive disorder via electrical stimulation. The utility of neural implants may be limited by the degradation of their performance in detecting neural signals over time. Such degradation may partly result from the brain's immune response, which is activated by penetration of the blood brain barrier. This response is characterized by immediate microglia/macrophage activation which signals increased upregulation of glial fibrillary acidic protein in astrocytes and formation of a glial scar that encapsulates the probe. However, the extent of this reaction varies greatly and is dependent on the size, shape, and insertion rate of the implant. According to some reports, this tissue response is coincident with a significant loss of neurons proximal to the device, but this mechanism remains unclear. To date, this putative neurodegenerative response has primarily been investigated through post-mortem labeling of neurons at different time points.
Here we report an in vivo optogenetic assay to detect changes in neuronal function and physiology throughout the course of the neuroinflammatory response to implanted devices. We used a chronic optogenetic stimulation protocol to provide repeatable controlled stimulation over many weeks of implanted time without susceptibility to electrode contact degradation mechanisms. Single-shank optrodes (Neuronexus Technologies, A16) were implanted into the primary motor cortex of adult male B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J mice. The probes were inserted so as to position the end of the fiber optic cable flush with the surface of the brain, and the recording sites throughout the cortical depth. After one week of recovery from implantation, weekly recordings were performed from awake, freely moving mice. Each session consisted of recording spontaneous activity for 10 minutes, followed by 10 minutes of optical stimulation with 5-100ms pulses at frequencies from 1-40 Hz. Delivered light was from a DPSS 473 nm laser with power emission of 1-50 mW. Electrophysiological recordings continued until 4 months post implant, or until discernible spikes could not be recorded. Preliminary data showed optically driven activity in both spiking and lower frequency bands. Properties of the spontaneous and optically-evoked activity were tracked longitudinally to assay for the presence of viable neurons, even in the absence of spontaneous or behavior-locked activity.

Disclosures: G.L. Knaack: None. **E. Civillico:** None. **K.T. Wachrathit:** None. **S. Huang:** None. **J.J. Pancrazio:** None. **C.G. Welle:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.05/GGG32

Topic: D.18. Brain-Machine Interface

Support: Interagency Agreement FDA-DARPA 224-10-6006

Title: Evaluation of chronically implanted microelectrode arrays for brain-machine interface

Authors: *P. TAKMAKOV[1], S. JAROUDI[1], E. F. CIVILLICO[2], K. T. WACHRATHIT[2], V. KRAUTHAMER[2], C. G. WELLE[2];
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Abstract: Neuroprosthetics with brain-computer interface provides an alternative motor functionality for paralyzed patients and amputees by directly linking the brain to artificial limbs via cortical implants that sample neural activity from a motor cortex. Currently, the chief roadblock for this technology is the decrease functionality of the implants with time. Experiments in primates and human patients have indicated degeneration of the device performance manifested as a decline both in amplitude and number of recorded neural spikes. It is unclear whether this degeneration pertains to the death or silencing of the adjacent neurons and encapsulation of the electrodes or to the failure of the implants due to chemical degradation. The neural implants are invasive high-risk medical deviceswich require extensive evaluation of their safety and efficacy is needed before this technology is common for the patients.
Electrochemical impedance spectroscopy (EIS) is the most efficient technique to assess the condition of the neural implant in vivo. Traditionally, only impedance at 1 kHz is reported and used to characterize the device. In this work, we evaluated the performance of cortical neural implants from several commercial manufacturers during chronic implantation in mice. Periodic recordings of action potentials along with EIS were performed for a period of up to one year. EIS recordings were conducted using wide band spectra from 1 Hz to 1 MHz to establish which frequencies best correlate with the observed decline in the electrophysiological signals. Our data indicates that using wide range of frequencies provides a more reliable prediction of the implant condition.
Additionally, we performed thorough characterization of the neural implants after they were removed from the animals. We developed a cleaning protocol based on mild cleaning agents including surfactants and digestive enzymes used to remove biological deposits. Characterization of the integrity of the neural implants was performed with EIS and scanning electron microscopy. In sum, evaluation of the electrode characteristics both in vivo and post-explant

elucidates associations between the functional decline in the device performance and the physical degradation of the implants.

Disclosures: P. Takmakov: None. **S. Jaroudi:** None. **E.F. Civillico:** None. **K.T. Wachrathit:** None. **V. Krauthamer:** None. **C.G. Welle:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.06/GGG33

Topic: D.18. Brain-Machine Interface

Support: DARPA N66001-12-1-4023

Title: A wireless neural recording system for freely behaving primates

Authors: F. ASGARIAN, M. HASHEMI, W. CARTAGENA, S. HAO, *K. G. OWEISS; Michigan State Univ., EAST LANSING, MI

Abstract: Wireless neural recording from awake behaving subjects has received much interest in recent years due to continued interest in improving our understanding of neural coding mechanisms outside of the pristine lab environments, and the urgent need for cosmetically acceptable solutions in clinical applications. Implantable microelectrode arrays that record spiking activity from ensembles of neurons, however, require ultra-high bandwidth links that preclude wireless telemetry with conventional electronics. A wireless system that enables high fidelity information transmission, while animals move freely in their natural surrounding is highly desirable. Here, we report on the first generation of our wireless, head-mounted neural interface for continuous intracortical neural recording from 96-channel Utah electrode arrays in primates. Besides being compatible with commercially available connectors/adaptors, the system has a configurable hardware platform, permitting calibration “on demand” depending on user-specific needs and experimental design.
The head mounted (transmitter) part consists of a Neural Interface Node (NIN) connected to the implanted array, while the receiver part consists of a Manager Interface Module (MIM) interfaced with a base workstation for programing and calibration purposes. The NIN processes neural data from 96 microelectrodes with a programmable sampling rate (up to ~23 KHz/channel) at 16-bit/sample resolution. The NIN further compresses and packetizes the data to enable wireless transmission over a 2-Mbps link (for each block of 32 channels) without compromising information about spike timing and identity on any given electrode. The system is battery powered, with a life time of 10~25 hours, depending on the enclosing case design. One

design features a case-battery (measures 35.5×53×24 mm^3), while another design features a backpack-battery with a subcutaneous cable extending to the skull (measures 50×54×36 mm^3). The MIM transfers data wirelessly to and from NIN, and is connected to the PC through USB. A Graphical User Interface (GUI) has been designed for display and programming purposes. The real-time processing capability of the system has been bench-tested with pre-stored monkey neural data. A fully implantable version is currently under development. Taken together, the system provides the most essential elements for recording from freely behaving subjects, and paves the way for fully implantable systems in Brain-Machine Interface applications.

Disclosures: F. Asgarian: None. **K.G. Oweiss:** None. **M. Hashemi:** None. **W. Cartagena:** None. **S. Hao:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.07/GGG34

Topic: D.18. Brain-Machine Interface

Support: NIH contract No: 1R01NS064318-01A1

DARPA contract No: N66001-06-C-4056

Title: Evaluation of long-term stability of atomic layer deposited Al2O3 and Parylene C bi-layer encapsulated Utah electrode array based neural interfaces

Authors: X. XIE[1], L. RIETH[1], L. WILLIAMS[3], *S. NEGI[1], R. BHANDARI[3], R. CALDWELL[2], M. DIWEKAR[1], R. SHARMA[1], P. TATHIREDDY[1], F. SOLZBACHER[1]; [1]Electrical and Computer Engin., [2]Bioengieering, Univ. of Utah, Salt Lake City, UT; [3]Blackrock Microsystems, Salt Lake City, UT

Abstract: Long-term stability and functionality of neural interfaces is a big challenge for chronic implantation. We present a novel encapsulation scheme that combines atomic layer deposited (ALD) Al2O3 and Parylene C for biomedical implantable. Our approach combines the highly effective moisture barrier properties of ALD alumina, and Parylene as a barrier to many ions and for preventing contact of alumina with liquid water. 52 nm of Al2O3 was deposited by plasma-assisted (PA) ALD on assembled Utah electrode arrays (UEAs) at 120 °C. A 6-µm thick Parylene-C layer was deposited by CVD using Gorman process on top of Al2O3 and A-174 (Momentive Performance Materials), an organosilane, was used as adhesion promoter. Hybrid methods were used to de-insulate the tips of the UEAs in order to interact with neurons for

recording and stimulation, including 200 laser pulses with fluence of 1400 mJ/ cm2, 2 minutes of oxygen plasma, and 8 minutes of buffered oxide etch etching. The tip exposure was ~ 35 µm. The devices were then put into saline solution for soak testing. The neural interfaces coated with 52 nm of Al2O3 and 6 µm of Parylene C were immersed in phosphate saline solution at 57 °C for accelerated lifetime testing. The median tip impedance of the bi-layer encapsulated wired Utah electrode array increased from 60 kΩ to 160 kΩ during the 960 days of equivalent soak testing at 37 °C. The loss of tip metal iridium oxide and etching of silicon in PBS solution contributed to the increase of impedance. Also bi-layer coated fully integrated Utah array based wireless neural interfaces had stable power-up frequencies at ~910 MHz and constant RF signal strength of -50 dBm during the 1044 days of equivalent soaking time at 37 °C. Bi-layer coated Utah arrays had steady current drawing of about 3 mA during 228 days of soak testing at 37 °C. The relatively stable tip impedance, constant power-up frequencies and signal strengths, and low current drawing suggested that the alumina and Parylene C bi-layer coating is very suitable for encapsulating chronic implantable devices.

Disclosures: X. xie: None. **S. Negi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Microsystems. **L. Rieth:** None. **L. Williams:** None. **R. Bhandari:** None. **R. Caldwell:** None. **M. Diwekar:** None. **R. Sharma:** None. **P. Tathireddy:** None. **F. Solzbacher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Microsystems.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.08/GGG35

Topic: D.18. Brain-Machine Interface

Support: FDA-DARPA IAA 224-10-6006

Title: A longitudinal study of comparative neural implant recording efficacy in awake behaving mice

Authors: *E. F. CIVILLICO, K. T. WACHRATHIT, A. JAIN, V. KRAUTHAMER, C. WELLE;
Office of Sci. and Engin. Laboratories, Div. of Physics, FDA, Silver Spring, MD

Abstract: The development of more reliable neural interfaces will benefit from a thorough characterization of the stability of electrophysiological features over lengthy chronic

implantations. Current intracortical neuroprosthetic systems transform the modulation of neuronal activity into command signals for prosthetic devices. Studies using both human and animal neuroprosthetic systems find a decline in quality of the recorded neural signal over time, reducing its information content for neuroprosthetic control. This decline may result from a complex tissue response, changes to the physical integrity of the electrode array, or a combination of both. To understand the factors determining electrode efficacy, we have developed a test platform consisting of a set of complementary surgical, recording, histological, and data analysis protocols for neural interface characterization in awake mobile mice. Microelectrode arrays are tested by surgical implantation, followed by biweekly 15-minute electrophysiological recordings from 2 weeks until 5 months postimplantation, followed by tissue sectioning and histology. Here we report results from various electrode form factors that are commercially available for research purposes, including silicon shank style arrays, microwire arrays, floating microelectrode arrays, and platinum-iridium arrays. All arrays were targeted to the motor cortex. A mixture of custom and off-the-shelf analysis routines was used to quantify activity. Measured quantities included RMS of the recorded signal from each site, amplitude and rate of multiunit activity, amplitude of putative single unit waveforms, number of single units identified, and stability of nonspiking signal components such as gamma-band power. Where appropriate, comparisons were made between different re-referencing methods and measurements are reported for multiple methods.
Preliminary analysis in a subset of recordings made with silicon probes showed declines in the multiunit event rate between 100 and 180 days postimplant. This decline did not track precisely with the amplitude of detected spike events. Immunohistochemical methods were used to examine the expression of foreign-body response-related markers such as GFAP (astrocytes), Iba-1 (microglia), and Nissl substance (neuronal somata), in the vicinity of the electrode. Preliminary histology data from mice successfully recorded for over 100 days showed minimal loss of neuronal cell bodies within 100 um of the electrode, as well as a mild microglia response. The results of these regulatory science studies will inform the evaluation of novel diagnostic and therapeutic medical devices in the CNS.

Disclosures: E.F. Civillico: None. **K.T. Wachrathit:** None. **A. Jain:** None. **V. Krauthamer:** None. **C. Welle:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.09/GGG36

Topic: D.18. Brain-Machine Interface

Support: National Science Foundation under Grant No. 0903622

Title: Keep your attention on the ball, a small twist on the old adage

Authors: *J. NORTON[1], S. HAAS[3], C. BESHERS[4], S. UMUNNA[1], T. BRETL[2];
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Abstract: In her collected works, "Perception, Cognition, and Decision Training", Joan Vickers reveals the importance of focusing on the ball immediately before a golf swing. Using eye tracking, Vickers is able to show that the series of steps leading up to a successful stroke culminates in a period of intense focus on the ball immediately prior to the backswing. In this brief moment, elite golfers focus their eyes directly on the point of contact between the ball and the putter. While the "quiet eye" has revealed a great deal about the sequence of events leading to a golf swing and the role of gaze, it has not dissociated gaze information from active attentional focus. Is the quiet eye simply a coupling of eye movements and motor activity or is there an attentional component? In order to investigate attention's role in performance, we use Steady State Visually Evoked Potentials (SSVEPs), a neural response that is known to be an index of attention. Results reveal that stroke performance is correlated with the allocation of attentional resources.

Disclosures: J. Norton: None. **S. Haas:** None. **C. Beshers:** None. **S. Umunna:** None. **T. Bretl:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.10/GGG37

Topic: D.18. Brain-Machine Interface

Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Multi-channel recording system with UWB wireless data transmitter for ECoG-BMI

Authors: *T. SUZUKI[1], H. ANDO[1], T. YOSHIDA[2], K. MATSUSHITA[3], M. HIRATA[3], T. YOSHIMINE[3], K. TAKIZAWA[1];
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Abstract: As a source signal for clinical Brain-Machine Interface (BMI), the electrocorticogram (ECoG) has recently attracted attention because of its good balance of features. It is less invasive but stabler than penetrating electrode methods and has a higher spatial resolution than the normal EEG. We have been developing a fully implantable human ECoG-BMI system that has 128 channels for clinical applications. To improve the accuracy of estimating motion intention and to achieve more precise control of a multi-joint prosthetic arm, it is necessary to increase the number of recording channels; however, there are several barriers, especially in the electrode array, amplifier, and wireless transmitting. In this research, we report a novel multi-channel recording system for ECoG based-BMI in which 1024 channels of ECoG signals are recorded, amplified, and transmitted wirelessly by UWB(Ultra Wide Band). In the proposed system, recorded signals are amplified and converted to digital (maximum sampling rate of the ADC is 1kHz per channel) by using a LSI chip, which has 64-ch low-noise amplifiers and ADCs. Then, the signals from 16 chips (1024-ch) are multiplexed by MUX units and transmitted wirelessly to the control unit located outside of the body by an UWB unit that is designed to use a frequency band that can be used internationally. The transmitting rate of the unit in the air is 133 Mbps. Evaluation experiments of the system with a body phantom system and animals are undergoing.

Disclosures: T. Suzuki: None. **H. Ando:** None. **T. Yoshida:** None. **K. Matsushita:** None. **M. Hirata:** None. **T. Yoshimine:** None. **K. Takizawa:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.11/GGG38

Topic: D.18. Brain-Machine Interface

Support: R44 NS061604-01A1

Title: Wireless electrocorticographic (ECoG) recording system

Authors: *D. R. MERRILL, R. ASKIN, C. F. SMITH, R. E. MADSEN, Jr., D. MCDONNALL, K. S. GUILLORY;
Ripple, Salt Lake City, UT

Abstract: We describe the design and development of a wireless recording system for short-term electrocorticographic (ECoG) monitoring. Reliable monitoring is necessary to guide accurate resection of cortical seizure foci in epilepsy patients who do not respond to drug therapies, but who are candidates for surgical treatment. State-of-the-art monitoring relies on percutaneous leads which may be a source of infection, and relatively stiff electrode arrays which may cause

hemorrhaging and unacceptable foreign body response. Percutaneous leads and stiff electrodes together have hindered the widespread deployment of this otherwise meaningful tool, resulting in only 0.5% - 1% of surgical candidates receiving treatment. The system described here comprises a wireless transcutaneous system using infrared data transmission which mitigates infection risk, and a manufacturing system for production of highly flexible electrode arrays which mitigates the foreign body response of traditional electrodes, together allowing the appropriate substantially larger epilepsy patient base to receive surgical treatment. Although the system design targets an underserved epilepsy population, the solutions offered by these technologies are also well-suited to providing a long-term brain-machine interface.
The flexible electrode manufacturing process is based on robotic deposition of alternating layers of nonconductive polymers, and polymers doped with electrically conductive particles forming the electrodes and interconnections. This process facilitates production of composite structures with trace and space pitches down to 300 μm, and supports synthesis of the electrode array and encapsulation of integrated electronics at low cost. We have performed flexural testing on electrodes, demonstrating electrical and mechanical reliability for over 14 million flexions. Signals from the flexible electrodes are routed to a subcutaneous electronics package where they are amplified and digitized. Data from the implant are then transmitted transcutaneously from an infrared emitter to a detector in the external transceiver. Robust data transmission is supported at rates of at least 10 Mbit/s with a power efficiency of 38% or greater. The implant is inductively powered from the external transceiver. Optimization of the coil driver circuit allows low voltage and low power operation, minimizing interference of the RF field in the optical detector.

Disclosures: D.R. Merrill: A. Employment/Salary (full or part-time):; Ripple. **R. Askin:** A. Employment/Salary (full or part-time):; Ripple. **C.F. Smith:** A. Employment/Salary (full or part-time):; Ripple. **R.E. Madsen:** A. Employment/Salary (full or part-time):; Ripple. **D. McDonnall:** A. Employment/Salary (full or part-time):; Ripple. **K.S. Guillory:** A. Employment/Salary (full or part-time):; Ripple.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.12/GGG39

Topic: D.18. Brain-Machine Interface

Support: ARO W911NF-11-1-0307

NIH 65-6052

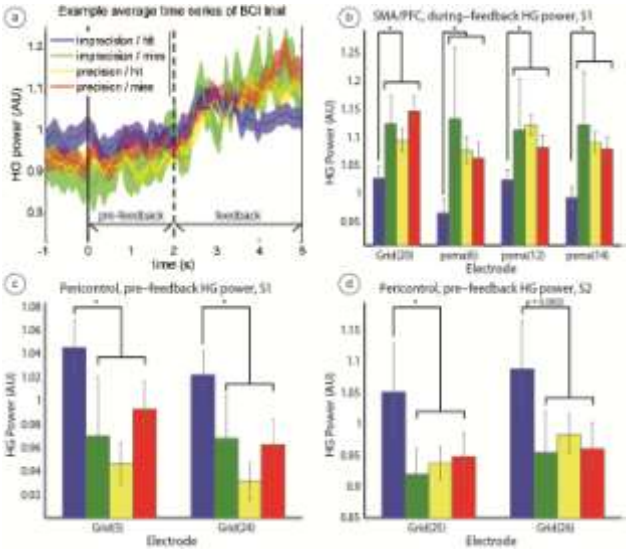
NIH NS065186-01

Title: Cortical representation of cognitive load in a graded-difficulty BCI task

Authors: *J. D. WANDER[1], T. BLAKELY[1], J. G. OJEMANN[2], R. P. N. RAO[3];
[1]Bioengineering, [2]Neurosurg., [3]Computer Sci., Univ. of Washington, Seattle, WA

Abstract: When executing motor actions, the nervous system dynamically modulates a wide variety of control parameters based on the expected precision requirement of the task being executed. To perform effectively in the face of diverse task requirements, brain-computer interfaces (BCIs) must modulate control parameters in a similar fashion, but without direct knowledge of the task being executed, cues for this modulation may need to be derived from neural activity. Two electrocorticography subjects were trained on a simple 1-D BCI task and then presented with a variant of that task involving targets that either did or did not require precision control. The amplitude of activity in the high-gamma band (HG, approx. 70-100 Hz) recorded at a single electrode over primary motor cortex was mapped to cursor velocity in the control dimension. In both subjects, we observed increases in HG activity near the controlling electrode in the pre-feedback task phase (two-sample t-test, $0.0012 < p < 0.0622$). In S1, we observed decreases in HG activity in pre-frontal cortex (PFC) and supplementary motor area (SMA) only when the precision requirement of the task was low and trial success was imminent ($0.0005 < p < 0.001$). These preliminary data suggest that, there exist neural correlates of both pre-trial and in-trial cognitive engagement corresponding to successful precision control of a BCI that could be leveraged in closed-loop BCI architectures to modify control parameters in real-time.

Caption:
Time series of average HG during trial. Target is presented at t=0, feedback begins at t=2. Source electrode was from S1, located over PFC. SEM shown in transparent color about the mean. (b) Comparison of HG activity for select electrodes from S1 during the last second of feedback for the four trial classes. PMSA (6, 12, 14) are located over SMA, Grid (20) is located over PFC. (c-d) Comparison of HG activity for select electrodes from S1 (c) and S2 (d) during the pre-feedback period. Grid (5, 24) for S1 and Grid (25, 26) for S2 are located over premotor and motor cortices.



Disclosures: J.D. Wander: None. **T. Blakely:** None. **J.G. Ojemann:** None. **R.P.N. Rao:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.13/GGG40

Topic: D.18. Brain-Machine Interface

Support: NIH 1R01NS064318-01A1

Title: Performance improvements in fully-integrated small form-factor wireless neural interfaces

Authors: *L. RIETH[1], D. WARREN[2], A. SMITH[4], R. HARRISON[5], P. TATHIREDDY[3], X. XIE[3], H. OPPERMANN[6], G. CLARK[2], F. SOLZBACHER[3];
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Abstract: The Integrated Neural Interface Program (INIP) has continued to make significant progress on our fully-integrated wireless neural interface system in terms of device lifetime, telemetry performance, and performance of the ASIC to accurately detect neural signals. Significant in-vivo recordings will also be presented to demonstrate the overall efficacy of the system. We have previously reported the improved performance of the 9th (INI-R9) version of the wireless ASIC, which demonstrate improved spike detector stability, better telemetry reliability, robust command reception, and a 2-level spike detection system. This report presents

the in-vitro and in-vivo performance characterization of the fully integrated devices, that includes fully wireless powering of the device through the integrated coil, and wireless telemetry of the recorded signals.
The integrated system consisted of a modified Utah Electrode Array (UEA) designed to be flip-chip bonded with the INI-R9 ASIC, capacitors, and gold coil, and be the platform for the encapsulation. The INI-R9 ASIC was developed with a 0.35 µm CMOS process fabricated using the X-Fab process. The ASIC utilizes a frequency-shift keyed (FSK) telemetry radio operating in the 910 MHz ISM band, with an aggregate bandwidth of 345 kbps, to transmit the spike-detected and single full bandwidth data streams. The ASIC is powered and data is telemetered to the device through a 2.765 MHz carrier that is amplitude-shift keyed (ASK). The integrated 5.5-mm diameter gold coil has an inductance of ~25 µH, and received power from 25 mm Class-E driving coil. The system was encapsulated using novel atomic layer deposited alumina layer, in conjunction with the Parylene and silicone encapsulation that has been previously reported. Details of the performance of this encapsulation are reported in a separate poster (Xie et al.)
This ASIC includes a diagnostic register to detect errors in the received commands, and was used to assess the command reception reliability. The command reception performance depended on position of the driving coil, medium surrounding the devices, and the characteristics for the amplitude modulation utilizes. Typical conditions used a 15% signal modulation. The command reception reliability of >90% could be achieved for testing in air or in PBS solution under optimized conditions. We achieved a typical bit-error-rates of 10^{-3}, and could achieve < 10^{-4} under optimized conditions. The recording performance of these devices in a feline peripheral nerve will be collected and analyzed as part of this poster presentation.

Disclosures: L. Rieth: None. **D. Warren:** None. **A. Smith:** None. **R. Harrison:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intan Technologies, LLC. **P. Tathireddy:** None. **X. Xie:** None. **H. Oppermann:** None. **G. Clark:** None. **F. Solzbacher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Microsystems.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.14/GGG41

Topic: D.18. Brain-Machine Interface

Title: Assessing awareness after traumatic brain injury (TBI) using spatially-constrained independent component analysis (ScICA)

Authors: *D. GUPTA[1,2], G. SELIGER[3,4], G. FIORENZA[3], D. ZEITLIN[3], B. ZOLTAN[3], L. TENTEROMANO[3], T. M. VAUGHAN[1,3];
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Abstract: This study seeks to detect awareness in patients with a profound disorder of consciousness following traumatic brain injury. We are using an electroencephalography(EEG)-based brain computer interface (BCI) protocol to determine whether a spoken command produces an EEG response. The long-term goal is to determine the prognostic value of such an analysis and to evaluate BCI communication in these patients.
The patient is asked to imagine specific hand and feet movements while EEG is recorded from 32 locations. We assess correlations between the instruction and the amplitudes and scalp distributions of specific EEG rhythms.
EEG activity is assumed to reflect a finite set of separate independent CNS or artifactual sources (overlapping in space, frequency, and time). Spatial filters (e.g., common average reference (CAR), Laplacian) are often used to isolate these sources. The effectiveness of a given filter is affected by electrode number, source orientations, and spatial spread, and may vary across subjects.
We are applying spatially-constrained Independent Component Analysis (ScICA) to extract the underlying sources from the EEG. ScICA exploits prior knowledge (e.g., likely spatial or spectral characteristics of the sources). It imposes constraints on the model to guide the ICA solution toward the expected source. As used here, it relies heavily on expected spatial topography, and optimizes a penalty function to find sources that have the expected topographies.
We are analyzing data from 4 subjects 30-60 days after traumatic brain injury (JFK coma score ≤10). The ScICA algorithm has spatial constraints favoring smooth topographies centered at Cz (feet motor imagery), or at C3 or C4 (hand motor imagery). The coefficient of determination (R2) is calculated for specific 3-Hz frequency bands from 2-45 Hz. We compare R2 for ScICA, for CAR filtering, and for unfiltered (i.e., ear-referenced) EEG. Initial results suggest that ScICA may improve real-time detection of an EEG response in patients with traumatic brain injury who are otherwise unresponsive.

Disclosures: D. Gupta: None. **G. Seliger:** None. **G. Fiorenza:** None. **D. Zeitlin:** None. **B. Zoltan:** None. **L. Tenteromano:** None. **T.M. Vaughan:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.15/GGG42

Topic: D.18. Brain-Machine Interface

Support: NINDS R01NS075889

Title: Rotations of neural population state present in scalp EEG recordings during human movement

Authors: *K. NATHAN, H. AGASHE, J. L. CONTRERAS-VIDAL, Ph.D;
Univ. of Houston, Houston, TX

Abstract: A recent article by Churchland et al. (Nature 2012) proposed that motor commands may be represented in cortex by operating on a system in which the oscillation parameters (e.g. phase, amplitude) of the population response are functionally related to the behavioral and kinematic parameters. This dynamical model suggests that the neural responses might reflect the underlying dynamics of the system, and that previously believed tuning models are merely incidental effects. The new model also found robust rotational structure in the transformed principal components (termed the jPCs) of the population response in the motor cortex of nonhuman primates during movement, both rhythmic (as in walking) and non-rhythmic (during reaching). However, it has not been shown whether these oscillatory features are represented in human cortex, or whether the rotational structure can be obtained by means other than intra-cortical spike recordings. Here we present evidence of similar rotational structure in whole-scalp electroencephalography (EEG) recordings from humans during walking and reaching tasks. EEG data were previously recorded from healthy human subjects and subjects affected by chronic stroke performing various walking and reaching tasks, and were analyzed using the same methods outlined by Churchland et al. Plotting the top two jPCs against each other revealed continuous circular structure for walking, whose speed was correlated with the amplitude of the rotations; remarkably, this result was also evident albeit slightly distorted in the stroke subjects. The EEG recorded during reaching tasks also had the similar brief rotational structure as previously reported in primate studies. Studies involving EEG recordings have often been criticized for having insufficient information content, particularly for brain-machine interface applications, despite the great benefits of the signals being acquirable without invasive surgery. Showing that the parameters of the rotational structure generated from the dynamical model can be obtained non-invasively reaffirms the consistency of the model across different recording methods and species. Furthermore, the evidence of the structure within EEG supports the use of EEG as a source signal for decoding and BMI applications just as invasive recordings.

Disclosures: K. Nathan: None. **H. Agashe:** None. **J.L. Contreras-Vidal:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.16/GGG43

Topic: D.18. Brain-Machine Interface

Title: Large field study of ultra-low cost BMI using intention decoding from eye movements for closed loop control

Authors: *W. W. ABBOTT[1], A. A. FAISAL[2];
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Abstract: Developments in the field of brain machine interfaces (BMI) hold the hope to restore independence to patients with severe motor disabilities via neuroprosthetics. However, current technology is either highly invasive or suffers from long training times, low information transfer rates, high latencies and high clinical costs (Tonet et al., 2008, J. Neurosci. Methods). We propose a non-invasive and ultra-low cost alternative - intention decoding from 3D gaze signals (Abbott & Faisal, 2012, J. Neural Eng.). Eye movements provide a high frequency signal directly relevant for neuroprosthetic control and are retained by patients with serious motor deficiencies, paralysis and limb amputation (Kaminski et al., 2002, Ann. N. Y. Acad. Sci.; Kaminski et al. 1992, Ann. Neurol.). Our system allows read-out bit rates of 43 bit s-1, well beyond conventional BMIs (EEG 1.63 bit s-1, MEA 3.3 bit s-1, EMG 2.66 bit s-1 , making our task-level BMI suitable for closed loop control of neuroprosthetics (Tonet et al., 2008, J. Neurosci. Methods; Abbott & Faisal, 2012, J. Neural Eng.). In the present study we performed a large-scale field study (n=867) to determine if naïve subjects could use our BMI to compete in an arcade video game (Pong - computer tennis). Two arcade cabinets with our embedded BMI system were built that allowed members of the public to briefly calibrate and then to play a game of pong (first to 5 points), controlling the paddle using just their eyes. Their opponent was either an AI computer player or another human player using a conventional control pad (up-down) input. During game play, the eye position, gaze estimation, control position and game state were recorded. Following a 30 second calibration, games lasted 76±34 seconds and subjects successfully returned 6.5±6.2 shots against their opponent (mean ± standard deviation). The average score when the subjects lost was 0.7±1.1 compared to the opponent average losing score of 2.1±1.4. Surprisingly, 20% of players could actually beat their opponent, despite total naivety to gaze-based interfaces before the trial, showing the intuitive nature of our system's control. Our study demonstrates the straightforward applicability of using the eyes as a BMI compared to other non-invasive methods that require significant training such as EEG and EMG. We also propose the game pong as a benchmark for the BMI field as it is a well-known easily accessible video game, which unlike

many BMI demonstrations, requires real-time closed loop control, vital for subject acceptance in neuroprosthetic control.

Disclosures: W.W. Abbott: None. **A.A. Faisal:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.17/GGG44

Topic: D.18. Brain-Machine Interface

Support: NSF Grant 0966963

The Hartwell Foundation

Title: Brain-Muscle-Computer Interfaces: The effect of audio feedback

Authors: *I.-M. SKAVHAUG, C. DAO, B. VERNON, S. S. JOSHI;
Mechanical and Aerospace Engin., Univ. of California, Davis, Davis, CA

Abstract: We are developing a novel Brain-Computer Interface that we call a Brain-Muscle-Computer Interface (BMCI), in which participants learn to control a cursor on a computer screen by manipulating the surface electromyography (sEMG) at one single muscle site (i.e. two control channels achieved from one recorded signal). Two-dimensional control was achieved by simultaneously adjusting the partial power in two separate frequency bands. Through the aid of visual feedback of cursor position, participants demonstrated a gradual improvement in target-hitting accuracy throughout four one hour-long testing sessions. In the current pilot study we investigated whether participants would be able to perform the cursor-to-target task when the feedback is auditory rather than visual. Auditory feedback was presented in the form of two tones of different auditory frequencies, one to the right ear and one to the left ear. The tones indicated to the participants the proximity of the invisible mouse cursor to the target's x- and y coordinates, respectively, by sounding at increasing temporal rates the closer the cursor got to the target in each dimension. This means that the participants received two sets of one-dimensional feedback that they had to integrate in order to hit the targets on the screen. The preliminary results show that participants in the audio group could learn to control the BMCI following four testing sessions, although more work must be carried out in order to establish whether the performance is comparable to that of groups receiving visual feedback and a combination of auditory and visual feedback. Our observations thus far are promising as there are situations in

which visual feedback to BMCI users is not convenient or even possible - for example in cases where the user is visually challenged.

Disclosures: I. Skavhaug: None. **C. Dao:** None. **B. Vernon:** None. **S.S. Joshi:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.18/GGG45

Topic: D.08. Pain

Support: SC1NS078778

R25GM059994

Title: Activation of a Gq-coupled membrane estrogen receptor rapidly attenuates alpha 2-adenoceptor-induced antinociception via an ERK I/II-dependent, non-genomic mechanism

Authors: *D. M. HECKARD[1], S. NAG[2], S. S. MOKHA[2];
[2]Neurosci. and Pharmacol., [1]Meharry Med. Col., Nashville, TN

Abstract: Sex differences in pain and analgesia are known, however, underlying mechanisms still remain elusive. We reported estrogen-induced attenuation of α_2-adrenoceptor-mediated antinociception, however, selective contribution of membrane estrogen receptors (mER) and mER-initiated non-genomic signaling mechanisms remains unknown. Hence, we selectively targeted spinal mERs in ovariectomized female rats using E2BSA (membrane impermeable estradiol), PPT, DPN, G1 and STX, selective agonists at ERα, β, G-protein coupled receptor 30 (GPR30), and Gq-coupled mER (Gq-mER), respectively. The effect of mER activation on clonidine (an α_2-adrenoceptor agonist)-induced antinociception was determined using nociceptive tail flick test. Tail flick latencies (TFL) were quickly assessed and expectedly, significantly increased by intrathecal (i.t.) clonidine. While i.t. PPT, DPN, and G1 failed to significantly alter, E2BSA or STX rapidly and dose-dependently attenuated clonidine-induced increase in TFL. This effect was reversible by, ICI 182,780, an estrogen receptor antagonist. Inhibition of *de novo* protein synthesis using anisomycin failed to alter the effect of E2BSA or STX suggesting unlikely contribution of genomic mechanisms. Immunoblotting of spinal tissue revealed mER activation-induced increased phosphorylated extracellular signal regulated kinase (ERK) I/II but not protein kinase A (PKA) or C(PKC) levels. Finally, *in vivo* inhibition of ERK I/II with U0126 blocked the effect of STX and restored clonidine antinociception. We provide strong evidence that Gq-mER may solely mediate estrogen-induced attenuation of clonidine

antinociception via a rapid, ERK I/II-dependent, non-genomic mechanism. Gq-mER blockade strategies may improve analgesia in females. Moreover, widespread distribution of α_2-adrenoceptor and mERs implicate our novel findings to cognitive, cardiovascular, and reproductive functions.

Disclosures: D.M. Heckard: None. **S. Nag:** None. **S.S. Mokha:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.19/GGG46

Topic: D.18. Brain-Machine Interface

Support: NIH R25 NS079200

R01 NS065186

Title: Human electrocorticography based stimulation

Authors: D. K. SU[1], J. D. WANDER[2], L. A. JOHNSON[1], D. SARMA[2], *K. E. WEAVER[3], J. D. OLSON[4], E. E. FETZ[5], J. G. OJEMANN[1];
[1]Neurosurg., [2]Bioengineering, [3]Radiology, [4]Rehabil. Med., [5]Physiol. and Biophysics, Univ. of Washington, SEATTLE, WA

Abstract: Introduction:
Numerous experiments using non-invasive cortical stimulation have demonstrated transient increases in motor cortical plasticity and improved functional rehabilitation in humans. We examined the feasibility and the effects of performing repetitive human electrocorticography (ECoG) based stimulation in an activity-dependent manner.
Methods:
4 epileptic patients undergoing subdural ECoG grid implants were enrolled in this study. Electrical stimuli were triggered by spontaneous movement leading to upper extremity electromyographic (EMG) activity, or increases in ECoG high gamma (HG) power overlying motor cortex, exclusively. An FDA-approved EMG stimulator was used to provide 5000 stimuli over 30 minutes; afterwards, ECoG recordings were taken while the subject performed cued motor tasks. No subjects experienced any adverse events.
Results:
A consistent decrease in event-related high-gamma activity after conditioning was seen. One subject, who had received 2 sessions of 5000 stimuli, also demonstrated a broadband increase in

HG power and a decrease in low frequency band (LFB) power during the resting state. These spectral changes were more apparent at sites closer to the stimulated electrodes, and lasted for at least 30 minutes. No significant changes in resting motor thresholds or correlation values between stimulation sites and the rest of the grid were noted. Latencies calculated for EMG-trigger to stimulation pulse delivery were normally distributed with a mean of 31-32ms.
Conclusions:
These surprising results suggest that, with this stimulation paradigm, repetitive electrical stimulation caused blunting of the normal HG response seen during motor movement. In the subject with the sustained broadband resting state changes, the prolonged effects perhaps suggest physiologic phenomena such as changes in neuronal resistivities or local neurotransmitter levels, or potentially reflect local charge build-up on the ECoG grid. This experiment demonstrates that invasive activity-driven human cortical stimulation is safe, feasible to accomplish within the window for spike timing dependent plasticity, and underlines the need for further investigation.

Disclosures: D.K. Su: None. **J.D. Wander:** None. **L.A. Johnson:** None. **D. Sarma:** None. **K.E. Weaver:** None. **J.D. Olson:** None. **E.E. Fetz:** None. **J.G. Ojemann:** None.

Poster

567. **Brain-Machine Interface IV**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 567.20/GGG47

Topic: D.18. Brain-Machine Interface

Support: Braingain Smartmix Porgramme Netherlands

Dutch Technology Foundation STW, UGT7685

University of Utrecht

Title: Prelocalization of a bipolar electrode using fMRI enables a minimally invasive ECoG-based Brain Computer Interface

Authors: *N. F. RAMSEY, M. BLEICHNER, A. TORRES VALDERRAMA, Z. FREUDENBURG, B. VERWEIJ, M. VANSTEENSEL, E. AARNOUTSE;
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Abstract: Invasive Brain-Computer Interface (BCI) technology promises many advantages over non-invasive BCI, but general acceptance is dependent on a minimal invasive approach. We envision a minimally invasive BCI with one surface electrode recording ECoG. Two major

problems arise to implant such a BCI: 1) how to non-invasively localize the areas where electrodes are placed and 2) where to place a reference electrode. We have solved these problems by using an fMRI protocol to localize placement of a bipolar electrode.
Eight patients with intractable epilepsy who were scheduled for surgery preoperatively performed a calculation task while 3T functional MRI images were collected. fMRI data was analyzed with SPM8 using GLM analysis. After implantation of 80-120 surface electrodes, all patients performed a BCI cursor task, during which they used mental arithmetic to control the cursor. The feature selected for control was high gamma power (65-95 Hz).
Six of the patients had successful BCI control (> 85%) using a unipolar electrode over the dorsolateral prefrontal cortex (DLPFC), re-referenced to the common average of all implanted electrodes. We analyzed retrospectively whether we are able to select a bipolar electrode (i.e. an electrode pair with a maximum inter-electrode distance of a few centimeters) the signal of which would give comparable results to a unipolar electrode referenced to the common average in terms of their discrimination between mental calculation and resting brain activity (r-squared measurement).
The coordinates of the strongest activating voxels within a predefined Region of Interest (DLPFC) were identified. Bipolar electrodes closely matching the fMRI coordinates were selected. We found significant differences in high gamma power between mental arithmetic and rest using the selected bipolar electrodes with inter-electrode distances of 1-3 cm for all six patients.
The preoperative localization of a suitable bipolar electrode was validated in the remaining two subjects. The coordinates of a bipolar electrode were identified before BCI experiments by defining a line of 4 cm through the fMRI foci with strongest activation. The selected bipolar electrode gave a high performance on the BCI cursor control task in both patients (70-94% and 92% respectively.). Post hoc comparison between uni- and bipolar signals revealed that the pair chosen yielded the highest r-square during the task of all tested pairs and for all unipolar signals for those electrodes.
In conclusion, fMRI prelocalization of bipolar electrodes yields high performance in BCI control. This method paves the way for minimally invasive BCI implants.

Disclosures: N.F. Ramsey: None. **M. bleichner:** None. **A. Torres Valderrama:** None. **Z. Freudenburg:** None. **B. verweij:** None. **M. Vansteensel:** None. **E. Aarnoutse:** None.**Poster**

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.01/GGG48

Topic: E.01. Neuroendocrine Processes

Support: Pekary Trust

Title: Acceleration of TRH and TRH-like peptide release in rat brain and peripheral tissues during proestrus/estrus

Authors: A. SATTIN, *A. E. PEKARY;
Res., VA Greater Los Angeles Hlthcare Syst, LOS ANGELES, CA

Abstract: The two major estrogen receptors, ERα and ERβ, mediate reproductive, metabolic, behavioral, neuroprotective, cognitive, neuroendocrine, immunomodulatory, and neurodevelopmental functions of estrogenic steroids. ERβ mediates more of the cognitive and neuroprotective effects of estrogens and less of the more familiar reproductive, metabolic and secondary sexual responses mediated by ERα. ERβ-selective agonists for the treatment of major depression, PTSD, and other anxiety disorders are the focus of pharmacologic development and clinical testing. Women are at greater risk for these disorders. Estradiol and its metabolites contribute to the neuroprotective effects of this steroid class, particularly in men, due to local conversion of testosterone to estradiol in key brain regions which are predisposed to neurodegenerative diseases. We have used young adult female Sprague-Dawley rats to assess the role of TRH and TRH-like peptides, with the general structure pGlu-X-Pro-NH_2 where "X" can be any amino acid residue, as mediators of the neurobiochemical effects of estradiol. TRH and TRH-like peptides have neuroprotective, antidepressant, anti-epileptic, and analeptic properties. The levels of TRH and TRH-like peptides during proestrus and/or estrus in the 12 brain regions analyzed were significantly decreased 87 times but increased only 8 times when compared to the corresponding levels during diestrus day 1 or 2. These changes, listed by brain region in the order of decreasing number of significant decreases (↓) and/or increases (↑) , were: medulla oblongata (15↓), striatum (14↓), hypothalamus (13↓), entorhinal cortex (9↓,4↑), cerebellum (9↓,2↑), amygdala (10↓), hippocampus (8↓), posterior cingulate (6↓), nucleus accumbens (2↓,1↑), frontal cortex (1↓,1↑), piriform cortex (0), and anterior cingulate (0). In peripheral tissues peptide decreases (accelerated release) during proestrus and estrus were observed 35 times while increases, all within the pancreas, occurred 7 times: ovaries (15↓), adrenals (11↓), uterus (8↓), and pancreas (1↓,7↑). Fluctuations in the levels of TRH and TRH-like peptides in brain and peripheral tissues observed during the female estrous cycle are most likely the combined effects of altered posttranslational processing of precursor peptides and their release/clearance by the excitatory glutamatergic nerves of the central and peripheral nervous system. We conclude that these peptides may be downstream mediators of some of the therapeutic effects of estrogen.

Disclosures: A. Sattin: None. **A.E. Pekary:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.02/HHH1

Topic: E.01. Neuroendocrine Processes

Support: CONACyT-220291

UNAM-DGAPA-PAPIIT IN-218911.

Title: The suprachiasmatic nucleus regulates the ovarian steroid secretion and the ovulatory response in an asymmetric way

Authors: D. A. RAMIREZ, A. GONZÁLEZ, H. JIMÉNEZ, E. VIEYRA, L. MORALES, *R. DOMINGUEZ;
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Abstract: The disruption of the circadian system in the Hypothalamus-Pituitary-Ovaries axis may be a factor affecting fertility. The suprachiasmatic nucleus (SCN) of the hypothalamus represents the circadian pacemaker in mammals, participating in the regulation of preovulatory release of gonadotropins releasing hormone (GnRH), since neurons located in preoptic area (that contains the largest concentrations of GnRH cell bodies in the rat brain) are innerved by efferent projections from the SCN. In rats, the bilateral lesion of the SCN results in lower progesterone and estradiol serum levels, disruption of LH timing secretion and ovulation. In hamsters exposed to constant light, the asymmetric activation of the GnRH neurons of the preoptic area correlates with the asymmetric activity of the SCN. Such results support the idea that the activity of the oscillators in each SCN can be different. Present study analyzes the effects of the unilateral lesion of the SCN at the day of proestrus on steroid ovarian secretion and ovulation response. For this purpose, cyclic female rats of the CII-ZV strain at 11:00 hours on proestrus were subjected or not (control) to a left (SCN-L) or right (SCN-R) lesion of the SCN, left (Sham-L) or right (Sham-R) sham surgery. The animals were sacrificed one or 24 hours after surgery. At autopsy, we assessed progesterona and estradiol serum levels by RIA. Spontaneous ovulation was analyzed in those animals sacrificed 24 hours after surgery. Sham-surgery did not modify ovulation, but results in changes in the ovarian hormones secretion, which depends on the side on which the electrode is inserted, as well as post-surgery period. The ovulation rate by rats with lesion of the SCN-R was lower than in Sham-R (2/5 vs. 5/5) as well as the number of ova shed 2.5±0.5 vs. 5.7±0.3, $p<0.05$). One hour after the lesion of the SCN-R, the progesterona levels were higher than Sham-R (39.4±3.6 *vs.* 28.5±2.2 ng/ml, $p<0.05$), while it was lower in rats with SCN-L 24 hours post-surgery (8.9±1.2 *vs.* 14.1±1.4 ng/ml, $p<0.05$). The estradiol levels were higher in the animals with lesion SCN-R than sham-R one hour after surgery (185.0±34.6 *vs.* 117.8±9.5 pg/ml, $p<0.05$). Present results suggest that the SCN-R modulates in a stimulatory

way the ovulatory process by the ipsilateral ovary. It is possible that the increase in estradiol levels can be the cause of a possible decrease in LH levels due to the inhibitory effect of estradiol, and therefore a decrease in ovulation. In conclusion, the activity of SCN-R regulates ovarian functions at 11:00 hours of proestrus day, whereas the SCN-L plays such regulation at other hours of the day.

Disclosures: D.A. Ramirez: None. **A. González:** None. **H. Jiménez:** None. **R. Dominguez:** None. **E. Vieyra:** None. **L. Morales:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.03/HHH2

Topic: E.01. Neuroendocrine Processes

Support: CONACyT grant 225344

UNAM-DGAPA-PAPIIT IN211813.

Title: Ovulatory response and monoamines concentration in the celiac-superior mesenteric ganglia and ovaries in rats with polycystic ovarian syndrome

Authors: R. LINARES[1], G. ROSAS[1], M. I. NAVARRETE[1], M. E. AYALA[1], *C. MORAN[2], R. DOMÍNGUEZ[1], L. MORALES[1];
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Abstract: The polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women during their reproductive years. It is characterized by ovulatory failure, amenorrhea, increased plasma androgen concentrations, and lower plasma capacity for binding sex hormone and, higher concentrations of estrogens. The PCOS etiology has been associated with disorders at the hypothalamic-pituitary level. In the last decades, it has been shown that the development of the syndrome also involves the sympathetic nervous system. The ovaries of animals treated with estradiol valerate (EV) increased noradrenergic tone which depends on the integrity of the superior ovarian nerve (SON). In rats with PCOS induced by EV injection or cold stress exposure the tyrosine hydroxylase activity and noradrenaline (NA) levels in the celiac ganglion (CG) were higher than in control. The unilateral or bilateral section of the vagus nerve (VN) restores ovulation in rats with EV induced PCOS. The VN has synaptic connections with the neurons originating the SON at the CG. To test the hypothesis that there is a relationship

between the catecholaminergic and serotonergic system level of the CG and the ovaries in PCOS rats, we assessed the concentration of NA, dopamine (DA), serotonin (5-HT) and their metabolites in the CG and ovary of rats with PCOS, induced by EV or stress. 10 day-old female rats of the CIIZ-V strain were injected with corn oil (Vh) or 2 mg of EV. The rats exposure to cold stress were placed from 24 days of age in a room at 4°C for 3 h/d for 8 wk (monday to friday) until sacrifice at day 80 of age. The VE-treated rats did not ovulate (EV 0/11 vs. Vh 12/12). NA levels in the CG of EV injected rats was higher than Vh (7.3±1.5 ng/mg vs. 3.7±0.8 p <0.05). The monoamines levels in the ovaries were similar to control (1.4±0.15 ng/mg vs. 1.4±0.14 n.s). Ovulation rate was not modified by cold stress (stress 9/10 vs. control 9/9); 5-HT level in the CG was higher than in control group (7.4±1.2 ng/mg vs. 1.8±0.4 p <0.05), while the monoamine levels in the ovary were not changed (0.5±0.15 ng/mg vs. 0.4±0.1 n.s). Present results suggest that the difference in the ovulatory and monoaminergic response in the two models of experimental PCOS, results from different mechanisms triggering the development of the disease, which depends on the induction way.

Disclosures: R. Linares: None. **C. Moran:** None. **G. Rosas:** None. **M.I. Navarrete:** None. **M.E. Ayala:** None. **R. Domínguez:** None. **L. Morales:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.04/HHH3

Topic: E.01. Neuroendocrine Processes

Support: NAT MORC_2013

Title: The anatomy of celiac ganglion related with the ovaries in female rats

Authors: *C. F. PASTELIN[1], Y. TÉLLEZ[1], M. MUÑOZ[1], N. ROSAS[2], A. HANDAL[1], Y. CRUZ[3], C. MORÁN[1];
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Abstract: The main component of the autonomic innervation of abdominal organs is provided by the celiac ganglion (CG). The aim of this study was to describe in adult female rats the anatomical organization of the CG and to determine its targets. 12 urethane anesthetized adult animals were used. CG and its branches were dissected and digital photographs were taken. Using a stereoscopic microscope, the gross features of the CG and the distribution of its branches

were determined. Neurons in small plexus were determined by histological techniques. The results showed that the CG is bilateral. Right and left celiac ganglia are located between the celiac artery and the superior mesenteric artery. The right CG was found in two shapes; pear shape (67%) and banana shape (33%). Independently of the shape, seven branches emerged from this ganglion. One branch ran ventrally to the aorta and joined the suprarrenal ganglion. Three branches ran medially, between the superior mesenteric artery and the celiac artery, one of these branches innervated the liver and the stomach, the other two intestinal viscera. Another branch, the thickest one, ran above the celiac artery and connected to the left CG. Other branch ran anterior to the aortic artery and innervated the ipsilateral kidney, and another one innervated lymph nodes. No branch reached the ovary. The left CG had banana shape (100%). From a ventral view five branches were observed. The bigger one ran caudally to the inferior vena cava, behind the left renal vein, and reached a small nervous plexus attached to the inferior vena cava. From this plexus emerged the innervation of the ovaries. Our findings show that in female rats the CG is asymmetric and only the left CG innervates the ovaries. The posganglionic neurons controlling the ovarian function could be in the CG or in the plexus attached to the inferior vena cava.

Disclosures: C.F. Pastelin: None. **Y. Téllez:** None. **M. Muñoz:** None. **N. Rosas:** None. **A. Handal:** None. **Y. Cruz:** None. **C. Morán:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.05/HHH4

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant R01NS048476

NIH Grant R01DK084052

Title: Met-enkephalin inhibits dopamine neurons in the hypothalamic arcuate nucleus and depresses presynaptic input

Authors: *X. ZHANG, A. N. VAN DEN POL;
Dept. of Neurosurg., Yale Univ. Sch. of Med., New Haven, CT

Abstract: By inhibiting pituitary prolactin release, dopamine neurons in the hypothalamic arcuate nucleus play an important role in lactation. Met-enkephalin (mENK), an opioid neuropeptide synthesized both in the arcuate nucleus and other loci in the brain, activates both

mu and delta opioid receptors. Although previous studies suggested that delta opioid receptor agonists influence arcuate dopamine neurons, no direct evidence has been reported. Here, using whole-cell patch clamp recording in slices of the mouse hypothalamus, we studied the effects of mENK on the activity of arcuate dopamine neurons. First, we recorded dopamine neuron activity in current clamp and found regular burst firing with a frequency of 0.07 ± 0.01 Hz (n=9) with each burst consisting of 5-50 action potentials (mean burst frequency of 7.1± 1.3 Hz for each burst), similar to arcuate dopamine neurons in rats (Lyons et al,2010). mENK (3 μM) reduced the burst frequency to 0.003 ± 0.003 Hz (n=9; $p<0.01$) and hyperpolarized the resting membrane potential from -58 ± 2.0 mV to -71 ± 2.2 mV (n=9; $p<0.01$). In the presence of TTX in voltage clamp, mENK induced an outward current of 17.5 ± 2.3 pA (n=7) at a holding potential of -60 mV suggesting direct postsynaptic inhibition. We also studied the actions of mENK on synaptic currents. mENK (3 μM) decreased spontaneous EPSC frequency from 3.2 ± 0.7 Hz to 1.7 ± 0.5 Hz (54.0 ± 6.0 % of control, n=8; $p<0.01$) and decreased IPSC frequency from 3.4 ±1.2 Hz to 0.4 ± 0.2 Hz (28.0 ± 5.0 % of control, n=5; $p<0.01$). Taken together, our results suggest that both direct inhibition and indirect modulation of synaptic transmission are involved in mENK regulation of arcuate dopamine neuron activity.

Disclosures: X. Zhang: None. **A.N. van den Pol:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.06/HHH5

Topic: E.01. Neuroendocrine Processes

Support: DGAPA IN 218911-3

CONACyT 29238.

Title: The muscarinic receptor blockade of the right suprachiasmatic nucleus decreases of the ovulatory response of the left ovary

Authors: E. VIEYRA[1], D. A. RAMIREZ[1], *S. E. CRUZ-MORALES[2], R. DOMÍNGUEZ[1]; [1]BRRU, FES Zaragoza-UNAM, Mexico City, Mexico; [2]Psychopharmacol. Div. de Investigación y Posgrado, UIICSE, FES-Iztacala UNAM México, Edo. Mexico, Mexico

Abstract: The neural mechanisms regulating ovulation are under circadian control in many species. In mammals, a master pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus orchestrates circadian timing coordinating reproductively relevant neuroendocrine

events necessary to maximize reproductive success. The gonadotropin-releasing hormone (GnRH) system contains the same circadian clock "machinery" necessary to generate autonomous cellular oscillations, and these subordinate clocks likely mediate daily changes in sensitivity to SCN communication. The SCN receives information from various regions of the CNS, some of which are cholinergic. The bilateral lesions of the SCN abolish estrous cyclicity, the preovulatory LH surge and ovulation. This study was designed to analyze the effects of muscarinic receptor blockade with atropine (ATR) in the Left SCN or Right SCN, at 9:00 of diestrus 1 on ovulation and serum concentrations of progesterone (P_4). Adult female rats of the CII-ZV strain at 9:00 hours at the diestrous 1 were micro-injected with 0.5 µl of saline (Vh) or 62.5 ng of ATR, into the left SCN or right SCN. The animals were sacrificed in the morning in the expected day of estrous. The micro-injection of ATR in the Right SCN resulted in high P_4 level than Vh injected ones (ATR 8.53±1.3 vs. Vh 5.05±1.0, $p<0.05$). The ATR in the Left SCN did not modify P_4 levels. The ovulation rate was not modified by ATR microinjection, but the number of ova shed by the left ovary or rats with ATR microinjection into the Right SCN was lower than Vh injected animals (ATR 3.3±0.7 vs. Vh 6.0±0.8 $p<0.05$). Present results suggest that at 09:00 of diestrous 1 day the stimulation of muscarinic receptors in the right SCN, originates a signal to a neuronal pathway modulating the multisinaptic ovulation regulation on the contralateral ovary. It is possible that such information participates in the regulation of follicular growth and differentiation.

Disclosures: E. Vieyra: None. **S.E. Cruz-Morales:** None. **D.A. Ramirez:** None. **R. Domínguez:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.07/HHH6

Topic: E.01. Neuroendocrine Processes

Support: CONACyT grant 225347

UNAM-DGAPA-PAPIIT IN211813

Title: The acute steroidogenic response of the ovaries to the vasoactive intestinal peptide on proestrus day is modulated by the superior ovarian nerve

Authors: G. ROSAS[1], M. I. RAMIREZ[1], R. LINARES[1], R. CHAVIRA[2], *M. MARTINEZ-GOMEZ[3], R. DOMÍNGUEZ[1], L. MORALES[1];
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Abstract: The superior ovarian nerve (SON) provides catecholaminergic and peptidergic innervation to the ovaries. *In vitro*, the vasoactive intestinal peptide (VIP) stimulates progesterone (P4), testosterone (T) and estradiol (E2) release from rat granulosa cells and whole ovaries. 24 hours after the VIP injection into the ovarian bursa (OB) of diestrus-2 rats, the E2 secretion by the left ovary was higher than by the right one. On estrus the VIPergic stimulation of the left ovary resulted in lower T levels. The aim of present study was to analyze the acute effects of OB VIP injection at proestrus on P4, T and E2 secretion in untouched rats and with uni or bilateral section of the SON. Sixty day-old female rats of the CII-ZV strain, were subjected at 10.30 h of proestrus to the unilateral or bilateral injection of 20 µl VIP (10^{-6}M) or saline solution (SS) in the OB. Other rats were submitted to the unilateral or bilateral section of the SON [left (L-SON), right (R-SON) or bilateral (B-SON)] followed by the injection of VIP into the denervated or innervated ovaries. Rats were sacrificed 60 minutes after treatment. Steroid hormones serum levels were measured by RIA. Compared to the control group, the VIPergic stimulation of the left ovary increased T (177.4±15.8 *vs.* 126.1±14.4 pg/ml; $p<0.05$) and decreased E2 levels (104.2±8.2 vs. 173.5±15.7 pg/ml, $p<0.05$). The injection of VIP into the right ovary resulted in a lower T levels (130.8±15.2 vs. 204.6±15.3 ng/ml; $p<0.05$). The injection of VIP into the ovaries of rats with L-SON resulted in lower P4 levels than animals injected with SS (VIP left ovary: 22.6±1.9 *vs.* 34.0±2.6 ng/ml; VIP right ovary: 26.1±2.1 *vs.* 35.1±2.2 ng/ml; $p<0.05$) and higher E2 levels (VIP left ovary: 288.6±21.5 *vs.* 130.5±8.8 pg/ml; VIP right ovary: 142.2±13.2 *vs.* 97.2±10.8 pg/ml; $p<0.05$). The VIPergic stimulation of the denervated ovary of rats with R-SON resulted in an increased P4 levels (33.1±2.5 *vs.* 24.9±1.7 ng/ml; $p<0.05$). The injection of VIP into the both ovaries of rats with B-SON resulted in higher P4 and T levels (30.2±2.9 *vs.* 21.0±1.8 ng/ml; 286.2±36.7 *vs.* 171.7±15.2 pg/ml; $p<0.05$, respectively) and lower E2 levels (99.9±6.9 *vs.* 174.3±17.3 pg/ml; $p<0.05$). Present results suggest that in the proestrus day the ovaries have different sensitivity to VIP which is modulated by the SON. Also, support the idea of a neural communication between the ovaries through the SON.

Disclosures: G. Rosas: None. **M. Martinez-Gomez:** None. **M.I. Ramirez:** None. **R. Linares:** None. **R. Chavira:** None. **R. Domínguez:** None. **L. Morales:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.08/HHH7

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant R01

Title: Comparative distribution of GAD67 mRNA expression in the forebrain of prepubertal and adult female mice

Authors: *D. RATRA, C. F. ELIAS;
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Abstract: GABA release on to GnRH presynaptic terminals within the median eminence prevents an increase in amplitude and frequency of gonadotropin pulsatile release; the latter being required for puberty. GABA, however, depolarizes GnRH cells in pre and peri pubertal female mice, and with additional evidence in the literature supporting GABA's depolarizing effects on GnRH cells, GABA assumes a more complicated regulatory role on pubertal development. The first order neuronal networks from which this GABA regulation arises is not entirely known and in an attempt to narrow down candidate neuronal populations involved, *in situ* hybridization was used to compare expression of Glutamate Decarboxylase (GAD67) before and after puberty. GAD67 is a 67kd enzyme required for the biosynthesis of GABA from Glutamate and is a marker for GABAergic neurons. This comparison would likely reveal variable expression of GAD67 unique to the developmental age of the sample; supporting findings of longitudinal differences in GABA concentrations within the stalk of the median eminence of Rhesus monkeys before and during puberty. In addition, the revealed variable expression would reside within cell populations possibly involved in regulating GnRH activity during puberty. In our study, whole-brain series from two groups of female C57BL/6J mice were used; a group of pre-pubertal females aged 20-24days with absent vaginal opening and a group of diestrous females aged approximately 60 days with observed normal cyclicity. These series were exposed to ^{35}S radiolabeled cRNA probes for GAD67. GAD67 hybridization between both groups showed considerable similarity throughout the cortex, striatum and thalamus. Within the hypothalamus however several differences were identified. The anterodorsal pre-optic area from the diestrous series contained a bright hybridization pattern absent in pre-pubertal tissue. The suprachiasmatic nuclei and the adjacent anterior hypothalamic area from the pre-pubertal series showed brighter hybridization overall compared to diestrous tissue. Lastly, the dorsomedial nucleus from the pre-pubertal series showed an increased area of hybridization compared to diestrous tissue. These results confirm variable longitudinal GAD67 expression within the hypothalamus of developing female mice and have identified GABAergic cell populations possibly involved in the regulation of GnRH activity during puberty.

Disclosures: D. Ratra: None. **C.F. Elias:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.09/HHH8

Topic: E.01. Neuroendocrine Processes

Support: NICHD Intramural Grants

Title: Gonadotropin-releasing hormone induces dentin matrix protein 1 in adult rat female pituitary glands

Authors: *I. BJELOBABA[1], M. KUCKA[1], S. J. H. CLOKIE[2], D. C. KLEIN[2], S. S. STOJILKOVIC[1];
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Abstract: Hypothalamic gonadotropin-releasing hormone (GnRH) is the primary regulator of reproduction in vertebrates, acting via the G protein-coupled GnRH receptor (GnRHR) expressed in pituitary gonadotrophs to control synthesis and release of gonadotropins. Using next generation sequencing, we characterized the GnRHR-regulated gene network in pituitary cells. This revealed 83 candidate genes that were up- or down-regulated by GnRH. These include genes encoding intracellular proteins involved in signaling and cytoskeleton functions, plasma membrane proteins controlling ion fluxes, and extracellular proteins acting as agonists for numerous receptors. Most notably, GnRH treatment induced a ~ 600-fold increase in mRNA expression of dentin matrix protein-1 (DMP1), one of five members of the small integrin-binding ligand N-linked glycoprotein gene family. This response was seen in cycling female pituitary cells and was 20-fold smaller in pituitary cells from males. These and related studies suggested that sex-specific DMP1 response to GnRH is established during the peripubertal period. *In vivo* studies further indicated that pituitary DMP1-mRNA is elevated during late proestrus, suggesting to us that GnRH-induced ovulation and induction of *DMP1* are synchronized and perhaps functionally related. The presence of DMP1 protein in gonadotrophs was confirmed by dual labeling immunohistochemistry. *In vitro* studies further confirmed that DMP1-mRNA expression is mediated by the GnRHR, as indicated by effects of a GnRHR antagonist and that it is not elicited by other hypothalamic releasing factors or other known gonadotroph agonists. Cell signaling studies revealed that GnRH-induction of *DMP1* is mediated by the protein kinase C pathway and reflects opposing roles of ERK1/2 and p38 MAPK. These results open a new line of research on the GnRHR-regulated network. The evidence that DMP1 production in female rat gonadotrophs is controlled by the hypothalamus through the GnRHR signaling pathway raises intriguing questions about the intrapituitary and downstream effects of DMP1 and possible roles in reproductive function.
Supported by NICHD Intramural Grants.

Disclosures: I. Bjelobaba: None. **M. Kucka:** None. **S.J.H. Clokie:** None. **D.C. Klein:** None. **S.S. Stojilkovic:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.10/HHH9

Topic: E.01. Neuroendocrine Processes

Support: CNPq in Brazil

Title: Estrogen and food state modulate leptin action on the nitrergic system

Authors: *B. BORGES, C. R. FRANCI;
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Abstract: It is known that leptin acts on the reproductive axis, but gonadotropin-releasing hormone (GnRH) neurons do not express leptin receptor. Nitric oxide (NO) has been suggested as a possible mediator of this action of the leptin. Previous data from our laboratory showed that leptin acts directly on neurons that express neuronal nitric oxide synthase (nNOS) in some brain areas related to the control of reproductive axis. Moreover, estrogen may modulate leptin action and nitrergic system. The aim of this work was verify if estrogen and/or fasting modulate the leptin action on cells that express nNOS in the medial preoptic area (MPOA) and in hypothalamic nuclei: arcuate (ARC), ventromedial (VMH), lateral (LH), dorsomedial dorsal part (dDMH), dorsomedial ventral part (vDMH) and premammillary ventral (PMV). Methods:Wistar female rats were ovariectomized and received a cannula in the lateral ventricle. After two weeks, they were treated with estrogen (10μg) or oil (0.1ml) for three days before the experiment day. One group of animal was subjected to the fasting during 48h before experiments. In the day of experiment animals received leptin i.c.v. (3μg) or saline (control) and after 30 minutes they were perfused to remove the brain for immunohistochemistry of nNOS and phosphorylated signal transducer and activator of transcription (pSTAT3). Results: Estrogen increased the number of nNOS immunorreactive (nNOS-ir) cells in the MPOA, LH and vDMH in fed-, but not fasted-control animals. Leptin increased the nNOS-ir cells in the VMH and PMV in fed- and fasted-control animals treated with estrogen, and in the MPOA and vDMH only in fasted-animals treated with estrogen. Leptin increased pSTAT3-ir in all areas studied in both fed and fasted. Moreover, the interaction of estrogen and fasting potentiate the increase of pSTAT3-ir in the MPOA, ARC, VMH, dDMH, vDMH and PMV. Leptin increased the co-expression of nNOS and pSTAT3 in all hypothalamic regions studied in fasted-animals treated with either estrogen or oil.

However, in the MPOA this increase only occurred in fasted animals treated with estrogen. In fed animals treated with estrogen, leptin increased co-expression of nNOS and pSTAT3 only in the VMH, dDMH, vDMH and PMV. Conclusion: Our data suggest an interaction between estrogen and leptin to modulate the nitrergic system in the MPOA, vDMH, VMH and PMV in fasted animals and only in the VMH and PMV in fed animals. Morever, in fasted animals, estrogen potentiates leptin action in all studied areas, except LH. This potentiated action was co-localized in nNOS-ir cells mainly in the MPOA, DMH and PMV.

Disclosures: B. Borges: None. **C.R. Franci:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.11/HHH10

Topic: E.01. Neuroendocrine Processes

Support: EKG R00 HL096830

NIH T90 DE021990-02

Title: Identification and selective stimulation of hypothalamic corticotropin releasing hormone containing neurons expressing light sensitive channelrhodopsin-2

Authors: *S. W. HARDEN, E. G. KRAUSE, C. J. FRAZIER;
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Abstract: Corticotropin-releasing hormone (CRH)-containing neurons control activation of the hypothalamic-pituitary-adrenal axis as well as the expression of anxiety-like behavior, and consequently are heavily implicated in the onset of stress-related disorders. Despite the important role of CRH containing neurons in the stress response, little is known about their intrinsic properties or connections. In the acute slice preparation, reliable identification of CRH neurons has been complicated by the heterogeneity of neuronal phenotypes within brain nuclei expressing CRH neurons. The broad objective of this study is to validate and test a novel reporter mouse line in which the CRH gene drives expression of the red fluorescent protein, dTomato, conjugated to channelrhodopsin-2 (ChR2). Specific goals are to use of dTomato fluorescence to identify and record from CRH positive neurons, and to selectively depolarize CRH positive neurons with light to induce neurotransmitter release. CRH neurons containing ChR2 were identified in brain slices using epifluorescence microscopy. A custom built TTL controlled LED stimulator was used to activate ChR2 with a variety of light pulses and trains. Whole-cell patch

clamp recordings were employed to characterize the intrinsic properties of CRH positive and CRH negative neurons, and to quantify responses to optical stimulation. Somatic recordings of CRH neurons in current-clamp mode revealed action potentials elicited by rapid pulses of blue light. Light-induced currents in voltage-clamp mode were quantified with respect to intensity and pulse length. Light-induced presynaptic stimulation and release of neurotransmitter was assessed by bath application of glutamate receptor antagonists. CRH / ChR2 positive neurons are able to be reliably identified for physiological studies in the acute slice preparation. LED-based activation of ChR2 is appropriate and effective for depolarizing these neurons in the hypothalamus and promoting presynaptic neurotransmitter release in the amygdala. Success in these objectives indicates a new ability to reliably identify and selectively drive CRH positive neurons in brain circuits mediating stress responding. This approach may allow more detailed and mechanistic studies on the role of CRH neurons in the stress response.

Disclosures: S.W. Harden: None. **E.G. Krause:** None. **C.J. Frazier:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.12/HHH11

Topic: E.01. Neuroendocrine Processes

Support: R01MH077152

Title: Remote stress-free peripheral administration of an endocannabinoid receptor antagonist suggests both central and peripheral actions regulating hypothalamic-pituitary-adrenal axis activity

Authors: *R. J. NEWSOM[1], R. J. GARCIA[1], H. E. W. DAY[2], S. CAMPEAU[2];
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Abstract: We recently reported that intraperitoneal (i.p) injection of a CB1 receptor antagonist, AM251, resulted in c-fos mRNA induction in the basolateral amygdala (BLA), paraventricular nucleus of the hypothalamus (PVN), and the prefrontal cortex (PFC), as well as an elevation in plasma corticosterone (CORT). This was in distinct contrast to a lack of induction in several measures such as c-fos mRNA in regions including the lateral septum (LS), bed nucleus of the stria terminalis (BST), medial preoptic area (MPOA), anterior pituitary gland, and in plasma adrenocorticotropin hormone (ACTH). This evidence suggested that the endogenous cannabinoid (eCB) system mediates tonic inhibition of activity in some central regions associated with

hypothalamic-pituitary-adrenal axis regulation, but peripheral actions at the adrenal glands were not assessed and could not be ruled out. In addition the stress of the intraperitoneal (i.p.) drug injection may have contributed to some of the c-fos mRNA induction observed. To more clearly examine the putative contribution of central vs. peripheral drug effects, and to help minimize potential injection stress effects, adult male Sprague Dawley rats were surgically implanted with i.p. catheters.After recovery from surgery, rats were placed, in their home cages, inside acoustically-attenuating chambers to acclimate overnight, and their ip catheters were connected to a length of PE tubing exteriorized. The next morning, at the circadian trough of HPA axis activity, rats were remotely injected with AM251 (1 or 2 mg/kg) or vehicle (n=7-8 per group). Rats were sacrificed 1 hour after administration, and brains, pituitary glands, and adrenal glands were quickly excised and frozen. Ongoing analyses indicate that CB1 receptor mRNA is more robustly expressed in adrenal glands compared to pituitary glands, in a pattern that might support the elevation in plasma CORT, but not ACTH, previously reported after administration of CB1 receptor antagonist. Additional brain c-fos mRNA induction analyses suggest that the prior pattern observed with ip injection of the CB1 receptor antagonist was not dependent on the stress of the injections. Overall, these results suggest both central and peripheral endocannabinoid signaling contribute to HPA axis regulation.

Disclosures: R.J. Newsom: None. **R.J. Garcia:** None. **H.E.W. Day:** None. **S. Campeau:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.13/HHH12

Topic: E.01. Neuroendocrine Processes

Support: 2004 PA Dept of Health Tobacco Settlement Funds to MER

NIH grant MH28380 to RTR

Title: Estrous cycle effects on hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine by osmotic mini-pumps, and nicotine withdrawal by mecamylamine in female rats

Authors: *M. E. RHODES[1], M. UDDIN[1], T. E. KAROWSKI[2], R. K. CZAMBEL[2], R. T. RUBIN[3];
[1]St. Vincent Col., LATROBE, PA; [2]Ctr. for Neurosciences Res., Allegheny Gen. Hosp., Pittsburgh, PA; [3]Psychiatry, VA Greater Los Angeles Healthcare Syst., Los Angeles, CA

Abstract: Our laboratory is studying the sexual diergism of hypothalamic-pituitary-adrenal (HPA) axis responses to cholinergic stimulation and antagonism. We previously reported that HPA responses to nicotine (NIC) and NIC antagonists are sexually diergic: Female rats had higher HPA responses than did male rats following 1) single-dose NIC, 2) single-dose NIC following continuous NIC for two weeks (modeling NIC habituation in experienced tobacco users), and 3) single-dose mecamylamine (MEC) following continuous NIC for two weeks (modeling NIC withdrawal in experienced tobacco users). The present study extends these findings by determining the influence of estrous cycle stages on HPA responses in the females. Jugular vein-cannulated male and female rats were housed individually and administered 0.3 mg/kg free-base NIC in the three experimental conditions noted above. Estrous cycle stage was determined by light microscopy of daily vaginal smears stained with methylene blue to determine cell types and proportions. Females then were arranged into proestrous and estrous (PE) and diestrous and metestrous (DM) groups, reflecting animals with relatively higher and lower circulating gonadal hormones, respectively. Blood sampling occurred before and after drug administrations for the determination of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) by highly specific immunoassays.
Following single-dose NIC, PE females had higher ACTH and CORT responses than did DM females and males. In contrast, following single-dose MEC (without continuous NIC), DM females had higher ACTH and CORT responses than did PE females and males. Males and females as a single group both had reduced ACTH and CORT responses to NIC challenge following NIC habituation; within the females, the PE group appeared to be more sensitive to the habituating effects of continuous NIC than did the DM group. NIC withdrawal by MEC increased ACTH and CORT responses in both males and females; PE females had higher ACTH and CORT responses than did DM females and males.
These results highlight the influence of the estrous cycle on HPA responses to NIC: DM females were more resistant to NIC habituation than were PE females and males, whereas PE females were more sensitive to NIC withdrawal than were DM females and males. Quitting smoking appears to be harder for women than men, and we can hypothesize from our results that the reinforcing and withdrawal effects of NIC may change across the menstrual cycle. Studying the relationships among NIC, stress, and gonadal hormone cycles may aid in developing improved smoking cessation plans for women.

Disclosures: M.E. Rhodes: None. **M. Uddin:** None. **T.E. Karowski:** None. **R.K. Czambel:** None. **R.T. Rubin:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.14/HHH13

Topic: E.01. Neuroendocrine Processes

Support: DK56863

DK57900

U01CA141464

DK082480

MH069860

DK091425

1F32HD68103

Title: Neuroendocrine but not behavioral arms of the stress axis altered by gastric bypass in male rats

Authors: *B. E. GRAYSON[1], A. P. HAKALA-FINCH[2], M. KEKULAWALA[2], H. LAUB[2], A. E. EGAN[2], S. C. WOODS[2], J. P. HERMAN[2], R. J. SEELEY[2], S. C. BENOIT[2], Y. M. ULRICH-LAI[2];
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Abstract: Although the rates of metabolic syndrome (MetS) and stress-related psychiatric disorders continue to increase in the US, it is unknown whether they are causally related. It has been suggested that weight loss may be an effective strategy to treat not only MetS, but also accompanying stress-related psychiatric disorders. Unfortunately current pharmacologic treatments result in only small transient reductions in body-weight. Several bariatric surgeries, however, produce sustained body-weight reduction in obese individuals. The present work determined whether surgically-induced weight-loss affects neuroendocrine and behavioral indices of stress-related psychiatric disorders in a rodent model. Adult Long Evans male rats were maintained on either a low-fat chow (Lean animals) or a palatable high-fat diet (Obese animals) prior to surgery. Animals received either sham surgery (Sham), vertical sleeve gastrectomy (VSG) or Roux-en-Y Gastric Bypass (RYGB). Both VSG and RYGB lost approximately 15% of their body weight following the surgeries. RYGB had an overall increased basal tone of the HPA axis as indicated by elevated morning (nadir) non-stress plasma corticosterone levels, increased adrenal weight and reduced thymus weight. During an acute stress test, RYGB exhibited a dampened HPA responsivity, possibly due to increased negative feedback from the elevated basal glucocorticoids. RYGB also exhibited attenuated cFos expression in the paraventricular nucleus and prefrontal cortex. In contrast, anxiety-related behaviors in the elevated plus maze and depressive-like behaviors in the forced-swim test were unaffected by either VSG or RYGB. Collectively, although VSG and RYGB result in similar

metabolic improvements, they differentially affect the HPA axis, with RYGB exhibiting a phenotype similar to chronic stress exposure. Increased basal HPA tone following RYGB may have significant long-term consequences to overall health, and suggests that RYGB individuals may be at increased risk for pathology following subsequent chronic stress exposure.

Disclosures: B.E. Grayson: None. **A.P. Hakala-Finch:** None. **M. Kekulawala:** None. **H. Laub:** None. **A.E. Egan:** None. **S.C. Woods:** None. **J.P. Herman:** None. **R.J. Seeley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ethicon Endosurgery. F. Consulting Fees (e.g., advisory boards); Ethicon Endosurgery. **S.C. Benoit:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ethicon Endosurgery. **Y.M. Ulrich-Lai:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.15/HHH14

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant MH066958

NIH Grant MH069879

Title: Stress plasticity of the noradrenergic regulation of inhibitory synaptic inputs to CRH neurons of the hypothalamic paraventricular nucleus

Authors: *C. CHEN, J. G. TASKER;
Cell & Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: The hypothalamic-pituitary-adrenal axis is a main regulatory system in the response to stress. Corticotropin releasing hormone (CRH) neurons of the hypothalamic paraventricular nucleus (PVN) release CRH as an initial signal in the HPA response to stress. CRH neurons in the PVN are activated by ascending projections from the noradrenergic A2 cell group located in the caudal nucleus of the solitary tract. The noradrenergic inputs have important effects on the function of neuroendocrine and autonomic systems. The PVN is under robust GABAergic regulation. Our study focused on the noradrenergic regulation of GABAergic synaptic inputs to CRH neurons under control conditions and following acute and chronic stress. Using whole-cell patch clamp recordings in CRH neurons in brain slices from mice expressing eGFP in CRH neurons, we found that norepinephrine (NE) application gave rise to two effects on GABAergic synaptic inputs, eliciting both a facilitation and a suppression of spontaneous inhibitory

postsynaptic currents (sIPSC) in separate CRH neurons. The facilitatory response was mediated by α1-adrenoceptor activation and was sensitive to blockade of spiking with Tetrodotoxin (TTX); the NE-induced suppression of sIPSCs was mediated by α2-adrenoceptor activation and was TTX-insensitive. The facilitatory response was blocked by inhibiting postsynaptic G protein activity with intracellular application of the G protein blocker GDP-β-s, but the suppressive response was not. These results suggest that norepinephrine activates 1)α1-adrenoceptors located in the postsynaptic cell membrane and elicits the release of a retrograde messenger that activates presynaptic GABA neurons, and 2) α2-adrenoceptors located at the presynaptic terminals of GABA synapses and suppresses GABA release. We found that acute restraint stress (30 min) prior to preparation of the brain slices abolished the NE-induced facilitation of sIPSCs, while the NE-induced suppression of sIPSCs was left intact. Exposure to chronic variable stress (3 weeks, alternating stressors) prior to slices preparation, on the other hand, abolished both the facilitation and the suppression of IPSC by NE. Our findings reveal the mechanism of NE regulation of GABAergic synaptic inputs to CRH neurons, which may contribute to activation of the HPA axis in response to stress, and indicate robust plasticity of the NE regulation of CRH neurons under different conditions of acute and chronic stress. Stress-induced changes in the synaptic regulation of CRH neurons could lead to dysregulationof the HPA axis, and may contribute to various stress-associated disorders, such as major depression and anxiety disorder.

Disclosures: C. Chen: None. **J.G. Tasker:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.16/HHH15

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA grant R01 AG34103 to CJP

Title: Interactive effects of low testosterone and obesity on the central and peripheral nervous systems

Authors: *A. JAYARAMAN, D. LENT, C. PIKE;
Univ. So California, LOS ANGELES, CA

Abstract: The normal, age-related loss of testosterone has deleterious effects on brain, adipose, and other androgen-responsive tissues throughout the body. Consequences of low testosterone include increased risk for a range of health conditions, including Alzheimer's disease (AD), obesity, and type 2 diabetes (T2D). Interestingly, obesity and T2D are independent risk factors

for AD. We hypothesize that the effects of low testosterone independently and cooperatively affect development of these disorders. Understanding the interactive effects of low testosterone with metabolic disturbances may contribute to identifying preventive and treatment strategies for T2D as well as determining the consequences of such interactions on neural health. Inflammatory pathways represent one mechanism of interaction. Pro-inflammatory pathways play a key role in the pathogenesis of several diseases, including T2D and AD, and are regulated by sex steroid hormones, obesity, and other factors. Testosterone can attenuate inflammation in part by decreasing the expression of pro-inflammatory cytokines such as TNFalpha and IL-1beta. Conversely, high-fat diet is associated with activation of pro-inflammatory pathways. High-fat diet also induces obesity, promotes T2D, and is associated with decreased testosterone levels. As one approach to investigating these relationships, we compared the effects of diet-induced obesity on nervous system endpoints under normal and low testosterone conditions. In particular, we determined the effects of low testosterone levels and high-fat diet on (i) expression of pro-inflammatory cytokines (ii) metabolic indices of T2D, (iii) levels of reactive astrocytes, activated microglia, and macrophage infiltration, and (iv) neuron survival and neurite outgrowth. Our results show that low testosterone levels and high-fat diet significantly elevate blood glucose levels, reduce insulin sensitivity, and increase expression levels of TNFalpha and IL-1beta transcripts. In addition, we find that neurons exhibit reduced survival and poorer neurite outgrowth when co-cultured with glial cultures generated from high-fat fed animals in comparison to glial cultures from animals maintained on a normal diet. We also observe changes in the inflammatory pathways in the sciatic nerve in response to low testosterone and diet-induced obesity. These results demonstrate neuroinflammatory effects of high-fat diet, a relationship that is affected by testosterone levels. Together, our findings suggest that low testosterone and obesity are interactive regulators of neuroinflammation that may increase risk of downstream disorders such as T2D and AD.

Disclosures: A. Jayaraman: None. **D. Lent:** None. **C. Pike:** None.

Poster

568. **Hypothalamic, Neural, and Peripheral Regulation of the HPG and HPA**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 568.17/HHH16

Topic: C.07. Developmental Disorders

Support: KAKEN #24650199 and 23810025

Takeda Science Foundation

Life Science Foundation of Japan

Title: Hypothalamic dysfunction in 5q14 deletion syndrome

Authors: *Y. SAKAI[1], Y. MATSUSHITA[2], K. OHKUBO[3], T. HARA[3];
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Abstract: 5q14.3 deletion syndrome is a rare chromosomal disorder characterized by moderate to severe intellectual disability, seizure and dysmorphic features. We report a 14-year-old boy with 5q14.3 deletion syndrome who carries a heterozygous deletion of myocyte-specific enhancer factor 2c (MEF2C) gene. In addition to the typical neuro-developmental features of 5q14.3 deletion syndrome, he showed recurrent hypoglycemia, appetite loss and hypothermia. Hormonal loading tests using insulin, arginine, and growth hormone releasing factor revealed that growth hormone was insufficiently released to serum in response to these stimulations, disclosing the hypothalamic dysfunction in the present case. To uncover biological roles of MEF2C in the hypothalamus, we studied its expression in the postnatal mouse brain. Notably, neuropeptide Y (NPY)-positive interneurons in the hypothalamic arcuate nuclei highly expressed MEF2C. In contrast, the Rett syndrome-associated protein, Methyl-CpG binding Protein 2 (MECP2) was barely expressed in these neurons. MEF2C knockdown or overexpression experiments using Neuro2a cells revealed that MEF2C activated the endogenous transcription of NPY. Conversely, siRNA-mediated knockdown of MECP2 led to derepression of the Npy gene. Taken together, these data support the concept that MEF2C and MECP2 share common molecular pathways for homeostatic expression of NPY in the adult hypothalamus. We herein propose that individuals with 5q14 deletion syndrome may exhibit neuroendocrine phenotypes that overlap with those in Rett syndrome through smilar molecular mechanisms.

Disclosures: Y. Sakai: None. **Y. Matsushita:** None. **K. Ohkubo:** None. **T. Hara:** None.**Poster**

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.01/HHH17

Topic: E.01. Neuroendocrine Processes

Title: Effects ofperinatal sucrose administration in rats

Authors: *I. ZARCO DE CORONADO, S. MUUS-MENDOZA;
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Abstract: This study was carried out in order to determine the effects of perinatal sugar rich diet (prenatal day 7 to postnatal day 25 in dams and offspring Wistar rats. Primigest animals were receiving Purine Chow Fodent Diet 5001 and 20% sucrose solution. Control rats received just water. Sugar solution or water and chow consumption, and body weight were registered. At the end animals were sacrifized under anesthesia. Experimental dams ingested sucrose solution /chow in the respective proportion 81.51%/17.48% and control animals 32.34%/67.65%. The body weight of control mothers increase and postweaning decrease was respectively 31.1 1.41 /58.2 and experimental mothers 49.0/58.2. Experimental dams exhibit hepatosteatosis and increased retroperitoneal fat. At birth, control offsprings weighted 5.57 g, and experimental offsprings weighted 6.08 g (p=0.014). At postnatal 25th day, female control offsprings weighted 37.98 g and experimental offsprings 45.30 (p=0.001); and male control offsprings weighted 37.01 g, and experimental offsprings 46.98 g (p=0.001). These data suggest perinatal sucrose diet modifies neuroendocrine factors of the homeostatic energy system.

Disclosures: I. Zarco de Coronado: None. **S. Muus-Mendoza:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.02/HHH18

Topic: E.01. Neuroendocrine Processes

Support: NIMH Grant MH099625

NIAAA Grant AA017354

Title: Ovarian hormones, but not androgens, affect neuron and glia number in the medial prefrontal cortex

Authors: *W. A. KOSS, R. M. SADOWSKI, J. M. JURASKA;
Psychology, Univ. Illinois Urbana-Champaign, CHAMPAIGN, IL

Abstract: There are sex differences in the adolescent development of the prefrontal cortex of both rats and humans (Geidd et al, 1999; Markham et al, 2007). At the cellular level, our laboratory has found that female rats show a greater loss of neurons in the medial prefrontal cortex (mPFC) than males during the adolescent period. Additionally, the loss of neurons occurs in both the outer (layers 2/3) and inner layers (layers 5/6) of the female mPFC whereas males only lose neurons in the outer layers. These changes result in an adult sex difference in the overall number of neurons in the mPFC (males>females). Sex differences also appear after

puberty in the number of glia in the rat prefrontal cortex (Markham et al, 2007). To investigate the role of pubertal hormones in organizing the cellular composition of the mPFC, the gonads were removed in the current study in both female and male rats prior to puberty (postnatal day 20-22) and same-sex littermates received sham surgeries. The total number of neurons and glia in the mPFC were stereologically quantified using software from Microbrightfield Inc. Results revealed that females without ovaries had more neurons and glia than intact females in the mPFC and this result was also significant for layers 5/6, but not layers 2/3. In contrast, males castrated prior to puberty did not have altered neuron or glia number in any layer of the mPFC. These data indicate that pubertal androgens do not affect the development of the mPFC during adolescence; however, the ovarian steroids play a significant role in the decrease in the number of neurons and glia in layers 5/6 of the female mPFC.

Disclosures: W.A. Koss: None. **R.M. Sadowski:** None. **J.M. Juraska:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.03/HHH19

Topic: E.01. Neuroendocrine Processes

Support: Wellcome Trust

Medical Research Council UK

Title: Fetal Testosterone is associated with white matter volumetric sexual dimorphism in children

Authors: *A. N. RUIGROK[1], E. CHAPMAN[2], M.-C. LAI[1], M. V. LOMBARDO[1], B. AUYEUNG[1], J. SUCKLING[3], K. TAYLOR[4], G. HACKETT[5], E. T. BULLMORE[3], S. BARON-COHEN[1];
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Abstract: Testosterone is known to have organizational effects on brain structure and function in non-human species. Variation in fetal testosterone (fT) was recently found to predict regional

gray matter volume in pre-pubertal boys. In an extension to this line of research we investigate how variation in fT predicts regional white matter volume in the same sample of boys aged 8-11 years old. We hypothesized that volume of areas associated with fT level overlap with sexually dimorphic regions in a congruent direction (e.g. a positive correlation with fT overlaps with areas larger in males and a negative correlation overlaps with areas larger in females) in an independent group of children in the same age-range. Structural brain scans were conducted in twenty-eight boys (mean age 9.5 years) who are part of the Cambridge Child Development Project. Fetal testosterone was measured from amniotic fluid samples collected between 13 and 20 weeks of gestation (fT levels ranged from 0.25-1.70 nmol/L). Data from the NIH Pediatric Repository of children (101 boys; 116 girls) between 8-11 years old (mean age of both sexes 9.5 years) were used as an age-matched group to identify sexually dimorphic structures. SPM8 and DARTEL toolbox were used for data preprocessing. Fetal testosterone positively predicted volume of a white matter region encompassing the left internal capsule, cortico-spinal tract, cortico-ponto-cerebellar tract, and the long segment of the arcuate fasciculus. No negative correlation of fT with white matter was observed. Areas overlapping with the corpus callosum (anterior and bilateral posterior) were larger in males, whereas areas overlapping with bilateral internal capsule, cortico-spinal, cortico-ponto-cerebellar tracts and the inferior cerebellar peduncles were larger in females, as well as a bilateral area overlapping with the inferior longitudinal and inferior fronto-occipital fasciculi. These results are the opposite of what was hypothesized; the area positively correlated with fT in boys overlaps with a sexually dimorphic region larger in girls. These findings suggest that fT may act differently as an organizing mechanism in white matter than in gray matter in 8-11 year-old children. Future research will apply diffusion tensor imaging and tractography to examine the relationship between white matter microstructural properties and fT.

Disclosures: A.N. Ruigrok: None. **E. Chapman:** None. **M. Lai:** None. **M.V. Lombardo:** None. **B. Auyeung:** None. **J. Suckling:** None. **K. Taylor:** None. **G. Hackett:** None. **E.T. Bullmore:** A. Employment/Salary (full or part-time):; GlaxoSmithKline. **S. Baron-Cohen:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.04/HHH20-DP9

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant SC3GM102051

Title: Expression and regulation of sexually dimorphic genes in the developing mouse cortex and hippocampus

Authors: C. ARMOSKUS, D. MOREIRA, *H.-W. TSAI;
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Abstract: The cortex and hippocampus are important for the control of cognitive and social behaviors, many of which are sexually dimorphic. However, the mechanisms that mediate the development of the brain structures and neural circuits underlying these functional differences between the sexes have not been discovered. Using gene expression microarrays, we have previously identified several novel sexually dimorphic genes expressed in the neonatal cortex and hippocampus, including Klk8 and Mid1 genes. Klk8 gene is highly expressed in the hippocampus and encodes a tryptic serine protease called kallikrein related-peptidase 8, involved in activity-dependent synaptic changes, such as long-term potentiation. Mid1 gene is located in the pseudoautosomal-region of the X- and Y- chromosomes and encodes a microtubule-associated protein with E3 ubiquitin ligase activity to target proteins for degradation through ubiquitination. Using mice as the model, many studies have established the essential role of perinatal surges in testosterone secreted by the testes during late embryonic development and immediately after birth in masculinization of brain structures and behaviors. Since androgen receptor is highly expressed in the cortex and hippocampus, we thus hypothesized that perinatal exposure to testosterone was required to regulate transcription of Mid1 and Klk8 genes in the male cortex and hippocampus. To test our hypothesis, we treated timed pregnant female mice daily with of vehicle (0.05 ml sesame oil, sc) or testosterone propionate (TP, 100 μg) starting on embryonic day 16. On the day of birth, the cortex and hippocampus were collected from vehicle- and TP-treated, male and female pups (n=10 per group). Total RNA was extracted from these tissues, followed by reverse transcription with real-time polychain reaction (RT-qPCR) to measure expression of Mid1 and Klk8. We found a significant effect of sex on Klk8 expression in the cortex and hippocampus. In both vehicle- and TP-treated mice, males showed higher levels of Klk8, than females. Mid1 was expressed higher in females than males, but was not affected by TP treatment. Our results have demonstrated sexually dimorphic expression of Mid1 and Klk8 genes in the neonatal mouse cortex and hippocampus. Although these genes don't seem to be regulated by testosterone, their sexually dimorphic expression along with molecular functions might implicate their important roles in the control of brain sexual differentiation, which might be involved in the development of distinct cognitive behaviors and sex-biased diseases, especially those that involve the cortex and hippocampus.

Disclosures: C. Armoskus: None. **D. Moreira:** None. **H. Tsai:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.05/HHH21

Topic: E.01. Neuroendocrine Processes

Title: Species differences in the distribution of androgen receptor and sex differences in three newly identified species-specific clusters in the preoptic and anterior hypothalamic areas of the adult rats and mice

Authors: *M. R. JAHAN, K. KOKUBU, C. MATSUO, R. FUJINAGA, A. YANAI, T. WATANABE, N. TAKEMOTO, M. N. ISLAM, K. SHINODA;
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Abstract: Background: The preoptic and anterior hypothalamic areas (PO/AH) are important sex-steroid responsive brain regions regulating intrinsic programs relevant to homeostatic, neuroendocrinergic and instinct behavioral functions. Some species difference in morphology and physiology, however, has been suggested in sex-hormone-induced functions of the PO/AH, whereas detailed distributions of sex-steroid receptors have never been compared between rats and mice. Nevertheless, data obtained from knockout mice have often been uncritically referred to those accumulated in rats. The purpose of this study is to clarify the differences in distribution pattern of androgen receptor (AR) in the PO/AH between rats and mice of both sexes. Material and Methods: Expression of AR in the PO/AH was immunohistochemically compared between adult rats (Wistar, SD) and mice (C57BL/6, DBA/2j, Balb/c) of both sexes, using completely serial frozen sections of paraformaldehyde-fixed tissues. Results: AR expression was much stronger in males than in female of both rodents. In general, AR-immunoreactive (AR-ir) cells were more conspicuous in mice than rats in both sexes, particularly in the medial preoptic area (MPO), posterodorsal preoptic nucleus, dorsal PO/AH junction (DPAJ) and suprachiasmatic nucleus (SCN). Exceptionally, AR-ir cells were more densely concentrated in the sexually dimorphic nucleus (SDN) of the MPO and periventricular zone of the AH in rats than mice. In addition, we discovered two distinct rat-specific small AR-ir cell clusters in both sexes as the “rostral nebular island (RNI)” in the DPAJ and “caudal nebular island (CNI)” in the caudal level of AH, while they are never observed in mice. The RNI and CNI are also identified in Nissl staining and exhibit significant male-dominant sex differences in their volume and cell numbers. We also found a distinct mouse-specific AR-ir cell cluster in the AH as the “tear drop nucleus” (TDN) in both sexes. The TDN was inconspicuous in delineation in Nissl staining, but shows significant male-dominant sex difference in its volume and AR-ir cell numbers when compared between gonadectomized male and female mice after equivalent enhancement in AR expression by excessive dihydrotestosterone. Conclusion: Prominent species difference in AR expression was clarified and the species-specific, sexually dimorphic AR-ir cell clusters were newly identified. The present results might explain different androgen induced psychotic, behavioral and endocrinergic responses between two rodents, warning that data related to androgen-

sensitive functions obtained from the two different rodents might not directly be applied each other.

Disclosures: M.R. Jahan: None. **K. Kokubu:** None. **C. Matsuo:** None. **R. Fujinaga:** None. **A. Yanai:** None. **T. Watanabe:** None. **N. Takemoto:** None. **M.N. Islam:** None. **K. Shinoda:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.06/HHH22

Topic: E.01. Neuroendocrine Processes

Support: NIH 5R01AG021133

NIH P01-AG000001

NSF PHY-085545

Title: Microcolumnar properties show sexual dimorphism in areas 17 and 46 of monkey brain

Authors: *W. MORRISON[1], E. L. GIANNARIS[2,3], L. CRUZ[4], F. MORTAZAVI[3], B. URBANC[4], J. SANTOS[1], D. L. ROSENE[3], H. E. STANLEY[1];
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Abstract: Sexual dimorphism is a general feature of mammalian neuroanatomy, from classical differences in the hypothalamus and amygdala to more recent observations of dimorphic hemispheric asymmetry of hippocampal structure (Cahill 2006). An important feature of the cortex is its organization into vertical columns referred to as micro- or mini-columns. Various aspects of microcolumnar structure, such as relative density (strength), width, spacing and regularity, can be quantified using statistical density maps (Cruz et al., 2005). We investigated whether gender differences extend to microcolumns, as suggested by Casanova and Tillquist (2008). We examined area 17 and area 46, two cortical areas that have been shown to have gender-specific functional differences in humans as well as rats (Tranel et al., 2005; Juraska 1984).
From a cohort of 7 male and 10 female M. mulatta, ages 7.4-19.7 years, area 17 tissue sections were prepared using NeuN immunohistochemistry. Neuron locations were identified with an

automated recognition algorithm and microcolumnarity assessed using the statistical density map. Columnar organization was related to gender through the use of stepwise discriminant function analysis, which shows whether or not a structural variable is useful in classifying an animal as male or female. The same analysis was performed in area 46 using Nissl stained tissue from a cohort of 14 male and 8 female monkeys, ages 4.8-19.8 years, previously analyzed (Cruz et al., 2008). Significant sexual dimorphism was observed in area 17 and the ventral portion of area 46 but not in dorsal area 46. In areas 17 and ventral 46, the microcolumns of males appear to be regularly spaced, while those of females were significantly less regular in spacing. Additionally, in ventral area 46, females were observed to have stronger columns.
Finally, when the same methods are applied to an aged cohort (9 male and 12 female monkeys aged 20.4 to 32.3 years) results suggest that gender specific structural differences diminish with age.

Disclosures: W. Morrison: None. **E.L. Giannaris:** None. **L. Cruz:** None. **F. Mortazavi:** None. **B. Urbanc:** None. **J. Santos:** None. **D.L. Rosene:** None. **H.E. Stanley:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.07/HHH23

Topic: E.01. Neuroendocrine Processes

Support: NIEHS P20 ES 018163

EPA RD 83459301 Project 4

NIEHS T32 ES007326

Title: Exposure to bisphenol A during early development alters neuron and glia number in the prefrontal cortex of adult male, but not female, rats

Authors: *R. N. SADOWSKI[1], L. M. WISE[2], S. L. SCHANTZ[1,3], J. M. JURASKA[1,2];
[1]Neurosci. Program, [2]Psychology, [3]Comparative Biosci., Univ. of Illinois at Urbana-Champaign, Champaign, IL

Abstract: Previous work has shown that exposure to bisphenol A (BPA) during early development can alter sexual differentiation of the brain in rodents, although few studies have examined effects on areas of the brain associated with cognition. The current study examined if developmental BPA exposure alters total number of neurons and glia in the medial prefrontal

cortex (mPFC) in adulthood. Long-Evans hooded rats were bred in the laboratory, and pregnant rats were orally exposed to corn oil (vehicle), 4 µg/kg, 40 µg/kg, or 400 µg/kg throughout pregnancy. After parturition, pups were given daily oral doses of oil or BPA, corresponding to those given during gestation, from days 1-9. Brains were examined in adulthood (P140), and layers 2/3 and layers 5/6 of the mPFC were parcellated separately to determine volume. The number of neurons and glia in the mPFC were then quantified stereologically with the optical disector. Results indicate that males exposed to 400 µg/kg BPA had increased numbers of neurons and glia in layers 5/6. Although there were no significant effects of BPA in layers 2/3, the pattern of increased neuron number in males exposed to 400µg/kg was similar to that seen in layers 5/6. Interestingly, increased neuron number in the PFC has also been reported in autistic male children (Courchesne et al., 2011). No effects of BPA were seen in females or in males exposed to the other doses of BPA. This evidence suggests that males are more susceptible to the long-lasting effects of BPA on the mPFC.

Disclosures: R.N. Sadowski: None. **L.M. Wise:** None. **S.L. Schantz:** None. **J.M. Juraska:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.08/HHH24

Topic: E.01. Neuroendocrine Processes

Support: NIEHS P20 ES 018163

EPA RD 83459301 Project 4

NIEHS T32 ES007326

Title: Effects of exposure to bisphenol A during adolescence on neuron number and volume in the prefrontal cortex

Authors: *L. M. WISE[1], R. N. SADOWSKI[2], S. L. SCHANTZ[3,2], J. M. JURASKA[1,2]; [1]Dept. of Psychology, [2]Neurosci. Program, [3]Comparative Biosci., Univ. of Illinois, Champaign, IL

Abstract: Bisphenol A (BPA), an endocrine disruptor commonly used in a variety of consumer products, has been found to alter neuroanatomy in multiple brain areas. However, few studies have examined long-term effects on the prefrontal cortex, and there is a lack of studies that

address whether BPA exposure during adolescence influences prefrontal neuroanatomy into adulthood. In the current study, Long-Evans male and female rats were administered 0, 4, 40, or 400µg/kg/day BPA during adolescent development (postnatal days 27-46). All other sources of BPA exposure were eliminated for the lifespan of the subjects. In adulthood (postnatal day 150), the volume, neuron number, and glia number of the prefrontal cortex are being assessed. The volume of the frontal white matter is also being analyzed. Previous research from our laboratory has shown a sex difference in volume of the ventral medial prefrontal cortex and frontal white matter. Preliminary data in the current study finds an abolishment of the sex difference in the frontal white matter with adolescent BPA exposure (sex x treatment, p=.02). Volumes of the prefrontal cortex layers 2/3 and 5/6 also show a similar pattern towards abolishment of the sex difference. Neuron number is currently being assessed.

Disclosures: L.M. Wise: None. **R.N. Sadowski:** None. **S.L. Schantz:** None. **J.M. Juraska:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.09/HHH25

Topic: E.01. Neuroendocrine Processes

Support: NSF IOS-0956831

Title: The role of circulating androgens and the androgen receptor in sex differences in the mouse VMH

Authors: *J. L. BRUMMET[1], C. L. JORDAN[2], S. M. BREEDLOVE[2];
[1]Psychology, [2]Neurosci., Michigan State Univ., East Lansing, MI

Abstract: The ventromedial nucleus of the hypothalamus (VMH) is a sexually differentiated area of the brain that is rich in steroid receptors. We compared the VMH of males and females to an induced testicular feminization (iTFM) mouse, which is genetically male, but lacks a functional androgen receptor (AR). Mice were gonadectomized at postnatal day 60 (PN60), implanted with a Silastic capsule that was either blank (B) or contained testosterone (T), and sacrificed at PN90. Brains were frozen, sectioned, thionin stained, and analyzed using stereology to estimate regional volume of the VMH as well as soma size, and neuron number within the nucleus. Preliminary results indicate that the regional volume of the VMH in mice is independent of AR and is controlled by circulating T (mean T-treated volume=2.6 ± .05 x 10^8 um^3, mean B-treated volume =2.3 ± .05 x 10^8 um^3; *p*=.003). As our lab previously reported in rats, the

dorsomedial and ventrolateral subdivisions of the VMH in mice account for most of the sexual dimorphism in the nucleus. In addition, neuron number is greater in males and iTfms than females, suggesting a non-AR dependent sex difference in neuron number (mean neuron number for males = 20.7 ± .95 x 10^3, iTfms= 19.1 ± .65 x 10^3, females= 16.6 ± .69 x 10^3; p=.002). Neuronal soma size is regulated by circulating T, with soma size larger in T treated animals (mean for T-treated soma size= 88.80 ± 1.78 um^2, mean for B-treated = 81.74 ± 1.60 um^2; p=.001). These results suggest that volume and soma size in the VMH are dependent on circulating T, while the sex difference in neuron number is independent of both AR and adult levels of circulating T. Preliminary evidence from follow up studies suggests there is also a sex difference in astrocyte number in the mouse VMH.

Disclosures: J.L. Brummet: None. **C.L. Jordan:** None. **S.M. Breedlove:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.10/HHH26

Topic: E.01. Neuroendocrine Processes

Support: the Japan Society for the Promotion of Science 24590257

Title: Immunohistochemical localization of estrogen receptors α and β, progesterone receptor, and kisspeptin in the preoptic area of SF-1 knockout mice

Authors: *Y. IKEDA[1], T. KATO[2], M. KOMADA[1];
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Abstract: In mammals, brain sex differentiation is considered to depend upon estrogens formed via the neural aromatization of androgens secreted by testes during a critical period including several days before and after birth. The transcription factor, Steroidogenic factor 1 (SF-1, officially designated NR5A1), regulates steroidogenesis and is essential for development of adrenals and gonads. SF-1 knockout (KO) mice serve as an animal model that is not exposed to endogenous gonadal steroids during both prenatal and postnatal development, since they lack gonads from the beginning of the differentiation. We have used them for studying when and how gonadal hormones act on the formation of sex differences in the brain during early postnatal development. In this study, we rescued SF-1 KO mice by corticosteroid injection since they die shortly after birth due to adrenal agenesis, and analyzed the expression of several markers including two estrogen receptors α and β, progesterone receptor, and kisspeptin, in the two

representative sexually different neuronal nuclei in the preoptic area; the anteroventral periventricular nucleus (AvPv), which is larger in females than in males and has important roles in female-typical GnRH/LH surge, and the medial nucleus of the preoptic region (MPN), which is larger in males than in females and has important roles in male sexual behavior, in SF-1 KO mice and wild-type (WT) controls, at postnatal stages, postnatal day 0 (P0), P7, P14, and P21, using immunohistochemistry. The localization and the positive cell number for these markers of SF-1 KO male and female mice were similar to those of WT females, indicating that the two brain regions of SF-1 KO male mice are feminized. The results support the idea that perinatal gonadal hormones are required for masculinization of the brain. This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science to Y Ikeda (grant number: 24590257).

Disclosures: Y. Ikeda: None. **T. Kato:** None. **M. Komada:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.11/HHH27

Topic:

Support: Eunice Kennedy Shriver NICHD/NIH grant R01 HD065856

Eunice Kennedy Shriver NICHD/NIH grant U54-HD012303

NIH training grant T32 HD007203

Title: Sexual differentiation of kisspeptin neurons still occurs in mice lacking GnRH signaling but feminization is incomplete

Authors: *K. P. TOLSON, J. KIM, S. DHAMIJA, A. S. KAUFFMAN;
Dept. of Reproductive Med., UCSD, La Jolla, CA

Abstract: Kisspeptin, encoded by *Kiss1*, regulates puberty and reproduction. In rodents, one population of kisspeptin/*Kiss1* neurons resides in the hypothalamic AVPV/PeN region. AVPV/PeN *Kiss1* neurons are sexually-dimorphic (greater in females), yet the mechanisms regulating their development and sexual differentiation remain poorly understood. Although neonatal estradiol (E2) normally masculinizes the AVPV/PeN kisspeptin system, emerging evidence suggests that developmental E2 may also be important for feminization of kisspeptin cells. However, precisely when in development E2 promotes kisspeptin feminization is

unknown. Here, we determined whether *Kiss1* expression is permanently impaired in adult *hpg* females (lacking GnRH and gonadal steroids) under different E2 replacement paradigms, and whether E2 is needed during juvenile or pubertal periods for complete feminization of *Kiss1* neurons. We demonstrate that 1) *hpg* females, who normally lack E2 exposure, have reduced AVPV/PeN *Kiss1* expression, even after various durations (acute and chronic) of adulthood E2 treatment; 2) despite reduced *Kiss1* levels in *hpg* females, significant sexual differentiation of *Kiss1* still occurs in *hpg* mice; 3) exposing *hpg* females to E2 during the pubertal period does not rescue their lower adult *Kiss1* levels; and 4) in normal mice, removal of ovarian E2 before the pubertal or juvenile periods does not prevent complete feminization of AVPV/PeN *Kiss1* expression or LH surge generation, indicating that puberty is not a "critical period" for *Kiss1* development. Thus, while sexual differentiation does occur in *hpg* mice, feminization is not maximal, and E2 is needed sometime before juvenile development for complete feminization of AVPV/PeN *Kiss1* neurons.

Disclosures: K.P. Tolson: None. **J. Kim:** None. **S. Dhamija:** None. **A.S. Kauffman:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.12/HHH28

Topic: E.01. Neuroendocrine Processes

Support: PICT0040/2008

CONICET/PIP2065

Title: Prenatal exposure to the antiandrogen flutamide affects mesocorticolimbic dopaminergic system in rats

Authors: M. PALLARÉS[1], E. ADROVER[1], M. IMSEN[2], C. J. BAIER[3], *M. C. ANTONELLI[1];
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Abstract: We have previously demonstrated that prenatal stress (PS) can exerts an impairment of midbrain dopaminergic system (DA) metabolism especially after puberty, suggesting a particular sensitivity of DA system to variations in gonadal hormones peaks. We further demonstrated that the hypothalamic-pituitary-testicular axis status of male rats exposed to PS

was altered since age-dependent changes on the external genitalia, pituitary and testicular hormones profiles and spermatogenesis rate were found. PS was shown to disrupt perinatal testosterone surges and since the developing forebrain DA system was shown to be influenced by androgen exposure, the aim of this study was to evaluate if the prenatal administration of flutamide, an androgen receptor blocker, to pregnant rats during late gestation, might affect the dopaminergic system development. Pregnant rats were treated daily from gestational day 14th to 21st with either vehicle (VEH: 5% ethanol-propylene glycol) or 10 mg/kg flutamide (FLU). Our results show that FLU reduced anogenital distance of male progeny and induced a delay in the completion of testis descend in all evaluated ages in comparison to VEH rats. Moreover malformation of penis, cryptorchidisim and atrophied seminal vesicle were also observed on FLU offspring. Morphological studies in mesocorticolimbic DA areas revealed that FLU males presented a decrease in the number of MAP2 immunoreactive neurons in comparison with VEH, suggesting that prenatal FLU induce a reduction in the dendritic arborization of mesencephalic structures, impairing normal connectivity between areas. Our results demonstrate that prenatal androgen manipulation induce similar consequences to PS suggesting that one of the possible mechanisms of prenatal insults might be related to the alteration of the organizational role of androgens and the differential modulation of their activational role on brain development

Disclosures: M. Pallarés: None. **E. Adrover:** None. **M. Imsen:** None. **C.J. Baier:** None. **M.C. Antonelli:** None.

Poster

569. **Sexual Differentiation of Neuroanatomical Endpoints**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 569.13/HHH29

Topic: E.01. Neuroendocrine Processes

Support: FONDECYT Grant N° 111-21205

MSI Grant Nº P10/063-F

Title: Neonatal exposure to estradiol valerate increases dopamine content in nigrostriatal pathway during adulthood in the rat

Authors: *R. SOTOMAYOR-ZÁRATE[1,2], R. RIQUELME[1], P. ESPINOSA[1], A. DAGNINO-SUBIABRE[1], P. JARA[3], G. M. RENARD[1,2], G. CRUZ[1];
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Abstract: The specific windows of sensitivity correspond to periods in which certain stimuli can generate long-term lasting effects (Lucas A. 1991). Research in programming has been focused in the study of stimuli that affect sensitive periods of development such as prenatal and neonatal stage. We previously showed that exposure to estradiol valerate (EV) to female rats during the first 12 hours of life increased the content of dopamine (DA), serotonin (5-HT) and noradrenaline (NA) in ventromedial-arcuatus hypothalamus of the adult rat (Sotomayor-Zárate et al. 2011). Alterations in nigrostriatal pathway could be involved in brain sensitivity to drugs of abuse and may play a role in addictive process. Therefore, the purpose of this work was to determine the neurotransmitters changes induced by neonatal EV (0.1 mg/50µL s.c per rat) administration on nigrostriatal pathway of adult female rats. Sesame oil (50µL s.c. per rat) was administered in a control parallel group. For this purpose, rats were killed by decapitation at 60 days of age and substantia nigra (SN)-ventral tegmental area (VTA) and striatum were microdissected. Tissues were weighed and homogenized in perchloric acid and centrifuged at 4°C. The supernatant was cleaned through a filtration unit and injected in a HPLC coupled to electrochemical detection to determine the content of DA, NA and 5-HT.
We observed that neonatal EV administration produces a significant increase in DA content in striatum and SN-VTA. In addition, the content of NA increases in striatum but no changes were observed in SN-VTA. In contrast, the content of 5-HT decreases in striatum but no changes were observed in SN-VTA.
Altogether, our results show that neonatal exposure to EV permanently modified the content of monoamine neurotransmitters in nigrostriatal pathway of adult rats. This might imply that the estrogenized rats could have a greater susceptibility to the effects of drugs of abuse.

Disclosures: R. Sotomayor-Zárate: None. **R. Riquelme:** None. **P. Espinosa:** None. **A. Dagnino-Subiabre:** None. **P. Jara:** None. **G.M. Renard:** None. **G. Cruz:** None.**Poster**

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.01/HHH30

Topic: E.08. Biological Rhythms and Sleep

Title: Effects of alcohol consumption in sleep architecture of Mexican elderly patients: A retrospective study

Authors: *M. M. MELENDEZ[1], A. GALLEGOS-CARI[1], N. F. HERNÁNDEZ-LLANES[1], R. E. CAMACHO-SOLIS[1], U. JIMENEZ-CORREA[2], F. AYALA-GUERRERO[2], J. VELAZQUEZ-MOCTEZUMA[3], A. JIMÉNEZ-ANGUIANO[3];

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Abstract:
Introduction:Mexico City is one of the most populated cities in the world. According with the National Institute of Statistics, Geography and Informatics, elderly population, in this city, was estimated in half million people in 2010, equals to 7% of total population, and this percentage will growth up to 28% in 2050 (almost a million elderly people). Sleep disorders are common nosological entities found in general population. Sleep disorders like insomnia are common in general population and prevalence of insomnia has been estimates between 9.0% and 17.7%, In México, Téllez-López et al. (1995) reports a prevalence of 22% in adult population. Studies made in other countries show that, in elderly population, prevalence has been estimated 28% in the USA, 32.9% in China and 37% in Iceland. Until today, there are no studies about sleep architecture in elderly patients of Mexico City. Aging process has been related with a modification in sleep pattern, including a shift in circadian phase, a decrease in slow wave sleep and difficulties in sleep maintenance. Risk factors associated with insomnia are sex, age, psychiatric comorbidities (like depression) and stress. Daytime sleepiness, a marker for insomnia, is largely observed in elderly population and is a risk factor for cognitive decline, obstructive sleep apnea, among others. Substance use also is related with sleep quality. Is reported that benzodiazepines, caffeine, alcohol and tobacco modifies sleep patterns, and this effect is more pronounced in elder population. The objective of this study was to assess the effect of alcohol consumption pattern in sleep architecture in a sample of elder population of Mexico City. Method: A retrospective cross-sectional study of files of elderly patients of the Sleep Disorders Clinic of the National Autonomous University of Mexico (UNAM) was conducted. The files include sociodemographical data, Epworth Sleepiness Scale and polysomnography records. All files were evaluated and diagnosed with insomnia according with the ICSD-2 Classification. Statical analysis was made with SPSS. Descriptive statistics was obtained over the sociodemographical data, insomnia and no-insomnia groups were compared over sleep architecture, alcohol consumption and sociodemographical data. Odds ratio was estimated for risk measurement in alcohol consumption and sociodemographical data for insomnia. Results: Differences in sleep architecture and alcohol consumption were observed between the insomnia and no-insomnia groups.

Disclosures: M.M. Melendez: None. **A. Gallegos-Cari:** None. **N.F. Hernández-Llanes:** None. **R.E. Camacho-Solis:** None. **U. Jimenez-Correa:** None. **F. Ayala-Guerrero:** None. **J. Velazquez-Moctezuma:** None. **A. Jiménez-Anguiano:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.02/HHH31

Topic: E.08. Biological Rhythms and Sleep

Title: The relation between alcohol consumption pattern and sleep architecture in Mexican elderly patients

Authors: *A. GALLEGOS-CARI[1], N. F. HERNANDEZ-LLANES[1], R. E. CAMACHO-SOLIS[1], U. JIMENEZ-CORREA[2], F. AYALA-GUERRERO[2], J. VELAZQUEZ-MOCTEZUMA[3], A. JIMÉNEZ-ANGUIANO[3], M. A. MENDOZA-MELÉNDEZ[1]; [1]IAPA-DF, Mexico City, Mexico; [2]UNAM, Mexico City, Mexico; [3]UAM I, Mexico City, Mexico

Abstract: Introduction: In Mexico City was estimated half million of elderly people in 2010,equals to 7% of total population, and this percentage will grow to almost a million in 2050. According to the "Survey on psychoactive Substance Use in Older Adults of Mexico City" conducted in 2012, the prevalence of alcohol consumption in the last 12 months in elderly was 32.3%, 44.2% for male and 24.9% for female. It's known that use and abuse of substances like benzodiazepines, caffeine, alcohol and tobacco affects sleep, and this effect is more pronounced in elder population. Aging process has been related with alterations in sleep pattern, including a shift in circadian phase, a decrease in slow wave and REM sleep. Some risk factors associated with insomnia are sex, age and comorbidity with depression and anxiety disorders. Daytime sleepiness, a marker for insomnia, is largely observed in elderly population. Sleep disorders like insomnia are common in general population, and in México, it is reported a prevalence of 22% in adults but studies in elderly population have shown an estimated prevalence range from 28% to 37%. The objective of this study was to measure changes in sleep architecture of Mexican elderly patients and its association with alcohol use and abuse. Method: We conducted a prospective study of 30 elderly users of the Sleep Disorders Clinic of the National Autonomous University of Mexico (UNAM). The participants signed an informed consent, authorizing their participation in the study, and completed the "Survey on psychoactive Substance Use in Older Adults of México City", an instrument designed to collect sociodemographical data, alcohol and other substances use patterns, index of quality life and sleep quality Epworth Sleepiness Scale. All participants were evaluated and diagnosed according with the ICSD-2 Classification by polysomnography. Statical analysis was made with SPSS. We analyzed descriptive statistics over sociodemographical data. Student´s t test was applied to compare insomnia and no-insomnia groups, sleep architecture and sleep quality, patterns of alcohol consumption and sociodemographical data. We also estimated odds ratio to measure risk factors associated with alcohol consumption and insomnia. Results: Alcohol use and abuse is related with changes in quality of sleep and sleep architecture among elderly patients with insomnia disturbances.

Disclosures: A. Gallegos-Cari: None. **N.F. Hernandez-Llanes:** None. **R.E. Camacho-Solis:** None. **U. Jimenez-Correa:** None. **F. Ayala-Guerrero:** None. **J. Velazquez-Moctezuma:** None. **A. Jiménez-Anguiano:** None. **M.A. Mendoza-Meléndez:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.03/HHH32

Topic: E.08. Biological Rhythms and Sleep

Support: CREST/JST grant

Title: Neural substrate of rapid eye movements during REM sleep in humans: Comparison of cortical activation patterns among REMs and several types of waking saccades

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Abstract: Since the discovery of the relationship between rapid eye movements (REMs) and dream content, one important issue is functional similarity between REMs and waking saccades. This relationship suggests that REMs occur to scan dream images during REM sleep. Animal studies reveal that cortical and subcortical structures, particularly the pons, play crucial roles in the generation of REMs. In contrast, there are no detailed comparisons of the activated regions related to REMs and waking saccades in humans, although the measures of these eye movements such as peak velocity have been rigorously compared. If REMs are analogous to waking saccades, REMs should be regulated by saccade-related cortical regions with visual information. To test this prediction, we compared spatial activation patterns among REMs and three types of waking saccades.
Twenty male and three female healthy volunteers participated in a sleep experiment, and 14 of them also participated in a waking saccade experiment. To identify brain activity accompanied by REMs, we simultaneously recorded fMRI and EEG in the early morning while participants slept in the MR scanner. fMRI data were analyzed in an event-related fashion. Three types of saccades were used in the waking saccade experiment: 1) visually guided saccades; 2) voluntary saccades with visual information; and 3) voluntary saccades without visual information. We calculated the correlation coefficients of spatial activation patterns in several cortical and subcortical regions among REMs and the three types of saccades and compared the correlation

coefficients.
REM-related activation was observed in the frontal eye field (FEF), supplementary eye field (SEF), medial visual cortices, putamen, thalamus, and brainstem. All these regions, except the brainstem, were also activated to some degree in the three waking saccade conditions. Furthermore, spatial correlation analysis showed that activation patterns of the medial visual cortices were similar among REMs, the visually guided saccades, and the voluntary saccades with visual information. In contrast, the activation patterns of the SEF and those of putamen were similar between REMs and the voluntary saccades without visual information. Although several saccade-related cortical regions were also activated in REMs, the lack of parietal eye field (PEF) activation accompanied by REMs and the role of PEF in saccade regulation suggest that the neural substrate of REMs are not identical to those of waking saccades with visual information.

Disclosures: S. Kan: None. **T. Koike:** None. **M. Misaki:** None. **S. Miyauchi:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.04/HHH33

Topic: E.08. Biological Rhythms and Sleep

Support: Human Frontier Science Program

Israeli Center of Research Excellence

NINDS R01NS055185

Title: Shared patterns of cortical neuronal activity associated with rapid eye movements during wakefulness and sleep in humans

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Abstract: In wakefulness, rapid eye movements (REMs) direct our fixation and attention to specific parts of the visual scene, shaping visual perception and guiding behavior. REMs are also prevalent during REM-sleep, a state associated with dreams and vivid mental imagery, but their relation to cortical neuronal activity and dreaming remains unclear.
In this study, we took advantage of the unique opportunity to examine neuronal activity associated with REMs during sleep and wake in the same participants. Thirteen presurgical epilepsy patients underwent continuous monitoring, providing written informed consent as approved by UCLA's IRB. Full-night polysomnographic sleep studies included EOG, EMG, four scalp electrodes and continuous video monitoring. Depth electrodes were implanted, according to clinical criteria, in 129 sites in medial temporal lobe, parietal and frontal cortices, recording depth EEG, LFPs, and unit activity (n=600). REMs were detected semi-automatically by a computer algorithm followed by visual inspection, to minimize false detections and precisely pinpoint REM onsets.
On average, 2.6 and 4.4 REMs/min were detected in wake and REM-sleep, respectively, and their shape was indistinguishable in wake vs. REM-sleep. Occurrence in NREM sleep was not significantly different from zero, so we considered only wake and REM-sleep for further analysis.
Neuronal activity across multiple cortical and MTL regions was robustly modulated with REMs. Accordingly, most neurons (63% in wake, 52% in REM-sleep) showed robust reduction in firing rate (33% of maximal firing rate on average, $p<0.05$) immediately before REM onsets, at times when behavioral saccadic suppression occurs. Reduction in firing was followed by increased spiking activity (60% of maximal firing rate) peaking 300-400ms after REM onsets ($p<0.05$). In wakefulness, the exact timing of such increased activity exhibited regional variability supporting a cortical-hippocampal propagation, and such variability is currently under examination in REM-sleep. In parallel with modulations in unit activities, REMs in both wake and sleep were associated with a positive peak (evoked potential) in the depth EEG occurring immediately before REM onset, akin to PGO potentials observed in animal studies. Finally, some neurons (37% in wake, 48% in REM-sleep) increased firing in the few hundred milliseconds before REM onsets, and this effect was stronger in sleep.
Overall, the results indicate that REMs are associated with shared patterns of cortical activity across vigilance states in humans, suggesting that REMs 'reset' activity patterns (and possibly visual perception) in both wakefulness and sleep.

Disclosures: T. Andrillon: None. **C. Cirelli:** None. **G. Tononi:** None. **I. Fried:** None. **Y. Nir:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.05/HHH34

Topic: E.08. Biological Rhythms and Sleep

Support: NINDS R01NS055185 to G.T.

Human Frontier Science Program (HFSP) long-term fellowship to Y.N.

Israeli Center of Research Excellence (ICORE) to Y.N.

Title: Human behavioral lapses upon sleepiness correlate with local suppression of single-unit spiking activity and regional increases in LFP low-frequency oscillations

Authors: *Y. NIR[1,3], T. ANDRILLON[4], N. SUTHANA[5], C. CIRELLI[3], I. FRIED[5,2], G. TONONI[3];
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Abstract: Lack of sleep affects cognition, mood, and health, modulating brain activity and behavior. Recently, we showed that prolonged wakefulness in rats leads to regional neuronal inactivity, low-frequency (2-6 Hz) waves, and behavioral deficits. Here we investigated whether similar phenomena occur in humans using the well-validated Psychomotor Vigilance Task (PVT).

Ten presurgical epilepsy patients underwent continuous monitoring, providing written informed consent to participate as approved by UCLA's IRB. Participants performed a face/place categorization in a modified PVT (n=27 sessions) while images were presented infrequently (ISI=2-8s). Some session pairs (n=8) were conducted before/after full-night sleep deprivation conducted for clinical purposes, and before/after normal sleep (n=2). Depth electrodes (>100 sites) were placed according to clinical criteria and included regions in medial temporal lobe (MTL), frontal and parietal cortices, and some scalp EEG channels. Extracellular activity was recorded from 1457 units.

Subjects successfully performed the face/place task (95% correct). Reaction times included normally-distributed fast trials, and a tail of exponentially-distributed slow trials (lapses). Objective sleep pressure had a selective and robust effect on lapses. Accordingly, sleep deprivation increased lapses more than two fold ($p<0.05$) and time spent awake before each session predicted lapses ($p<0.01$). Subjective ratings of sleepiness were a more variable predictor. Controls argued against learning / motivation / circadian factors as affecting lapses.

Next, visually-evoked neuronal activity during PVT was examined. 96 neurons (18% of MTL

neurons) responded significantly to pictures. We compared neuronal activity during lapses vs. trials with identical images associated with fast correct behavioral responses. Importantly, focusing on correct trials rules out eye closure and micro-sleeps. During lapses, unit responses in multiple MTL regions exhibited a significant suppression ($p<0.01$) in firing rates 200-300ms after image onset. Such suppression was local - could not be detected in non-responsive MTL or frontal channels. Visual presentation also led to increased power in high (>40Hz) frequency LFPs and decreased power in low (<20Hz) frequencies. During lapses, low-frequency power was significantly increased, whereas high frequency power was significantly reduced - in line with suppression in spiking activity. LFP effects were likewise observed mainly locally in the MTL. These findings suggest that in humans, as in rats, "local sleep" during wakefulness may underlie the cognitive effects of sleepiness.

Disclosures: Y. Nir: None. **T. Andrillon:** None. **N. Suthana:** None. **C. Cirelli:** None. **I. Fried:** None. **G. Tononi:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.06/HHH35

Topic: E.08. Biological Rhythms and Sleep

Title: Fading signatures of critical brain dynamics during sustained wakefulness in humans

Authors: *C. MEISEL[1], E. OLBRICH[2], O. SHRIKI[1], P. ACHERMANN[3];
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Abstract: Sleep encompasses roughly a third of our lifetime, yet its purpose and biological function are not well understood. Without sleep optimal brain functioning such as responsiveness to stimuli, information processing or learning may be impaired. Such observations suggest that sleep plays a crucial role in organizing or reorganizing neuronal networks of the brain towards states where information processing is optimized.
Increasing evidence suggests that normal cortical activity operates near a critical state characterized by balanced activity patterns. Theory and experiment have shown that such critical dynamics optimize information processing.
However, it remains unknown whether critical dynamics is affected in the course of wake and sleep which could account for changes in information processing capabilities.
Here, we investigate the hypothesis that wakefulness moves cortical networks away from a

critical state which is restored by sleep.
We analyze EEG from eight healthy subjects during a 40 hour period of sustained wakefulness (sleep deprivation) and after recovery sleep.
We find the precise power-laws governing the cascading activity of neuronal avalanches and the distribution of phase-lock intervals (PLIs) under normal conditions to be increasingly disarranged towards a bimodal distribution during sustained wakefulness. Accordingly, we find the branching parameter to be close to 1 initially indicating that one event on average leads to one future event and to significantly increase to a value larger than 1 with growing sleep deprivation. We conclude that the decline of balanced activity indicates a progressive distance from criticality towards states characterized by an imbalance towards excitation where larger events prevail dynamics. Conversely, sleep restores the critical state resulting in recovered power-law statistics and branching parameter. These findings support the intriguing hypothesis that sleep may be important to reorganize cortical network dynamics to a critical state thereby assuring optimal computational capabilities for the following time awake.

Disclosures: C. Meisel: None. **E. Olbrich:** None. **O. Shriki:** None. **P. Achermann:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.07/HHH36

Topic: E.08. Biological Rhythms and Sleep

Support: 2R01EB009282

Title: Reproduction of whole-head MEG and EEG patterns during Human Sleep Spindles in a large scale neural model with realistic cortical anatomy

Authors: E. MUKAMEL[1], D. J. HAGLER JR.[2], G. P. KRISHNAN[3], E. PETRILLO[3], S. S. CASH[4], T. SEJNOWSKI[1], *M. V. BAZHENOV[5], E. HALGREN[2];
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Abstract: Sleep spindles are prominent thalamocortical bursts of 10-15Hz oscillations lasting ~1s. They have been implicated in memory and disease, and their neurobiology at a circuit, synapse and channel level have been extensively studied in animals. Spindles measured with the electroencephalogram are largely synchronous and distributed, whereas those measured with the magnetoencephalogram are largely asynchronous and focal, leading to the hypothesis that the

former are generated by the matrix thalamocortical system, and the later by the core. Here we test this hypothesis with a computational neural model containing both matrix and core elements in the thalamus and cortex. The thalamus implements all relevant intrinsic currents. The cortex includes 25,600 patches realistically arranged on the folded cortical surface. The cortical activity during modeled spindles is propagated to the EEG and MEG sensors. Three models are examined, a 'core model' with a single focal thalamo-cortical spindle generating system, a 'matrix model' with a single diffuse system, and a 'core/matrix model' with separate but interacting focal and diffuse systems. Only the core/matrix model reproduced the empirically observed topography, strength and coherence of EEG and MEG during spindles. The core/matrix model explicitly represents the interaction of focal and diffuse thalamo-cortical systems, with readily obtained empirical predictions at multiple levels. This integration of focal with distributed information processing may be a general mechanism of thalamocortical physiology.

Disclosures: E. Mukamel: None. **M.V. Bazhenov:** None. **D.J. Hagler Jr.:** None. **G.P. Krishnan:** None. **E. Petrillo:** None. **S.S. Cash:** None. **T. Sejnowski:** None. **E. Halgren:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.08/HHH37

Topic: E.08. Biological Rhythms and Sleep

Support: R01 MH099645

Title: Sleep stage transitions in the network model of the thalamocortical system

Authors: *G. P. KRISHNAN[1], E. HALGREN[2], M. BAZHENOV[1];
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Abstract: In the EEG, stage two of sleep is characterized by spindle activity (8-12 Hz) and the occasional K-Complex, while stage three or slow wave sleep is characterized by the slow oscillation (< 1 Hz). Activation of neuromodulatory systems controls transition between sleep states. An increase in the level of acetylcholine release associated with activation of nicotinic and muscarinic receptors depolarizes cortical pyramidal cells and thalamic relay cells both in awake states and during REM sleep. This effect is achieved mainly by blocking resting potassium conductances in these cells. Furthermore, in states of arousal when forebrain activity is high, intracortical synapses are relatively depressed via presynaptic GABA-B and muscarinic receptors. In this study, we show that a decrease in intracortical excitatory connections is sufficient for the transition from slow oscillation to stage two sleep. We demonstrate this using a

computational model of the thalamocortical network including core and matrix thalamic relay cells, reticular thalamic neurons and cortical layers. The network displayed stage two sleep activity with intermittent spindles at 8 Hz in thalamic and cortical neurons similar to experimental studies. External stimulation applied to thalamic reticular neurons led to isolated Down states - K-Complexes - in the entire thalamocortical network. Upon increase of the strength of excitatory intracortical connections, sleep slow oscillation like activity emerged in the network. The increase in excitatory connections was sufficient to induce active (Up) and silent (Down) cortical states which occurred synchronously across the network leading to the average activity around 1-1.5 Hz. The active states lasted for 500-600 msec, while silent states lasted for 100-300 msec. Increasing potassium leak conductance, an effect associated with decreased acetylcholine release during deep sleep, facilitated the transition to slow oscillations. The transition between sleep stages occurred abruptly in the model with a short period of mixed activity. Overall, this study confirms that the change in synaptic connection strength which is produced by activation of neuromodulatory systems can act as a primary mechanism for the transition between stage two sleep and slow wave sleep.

Disclosures: G.P. Krishnan: None. **E. Halgren:** None. **M. Bazhenov:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.09/HHH38

Topic: E.08. Biological Rhythms and Sleep

Support: US Public Health Service Grant R01 MH62521

Title: Longitudinal data reveal a linear increase in the frequency of peak sigma power across ages 6-18

Authors: *N. DARCHIA[1], I. G. CAMPBELL[2], I. FEINBERG[2];
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Abstract: Sleep and EEG change substantially across childhood-adolescence. NREM delta (1-4 Hz) power increases steeply in infancy to a maximum in mid-childhood and then begins a slow decline that accelerates sharply at age 12, falling by about 60% in the next 5 years. NREM theta power begins age decline earlier than delta but its decline also accelerates sharply between 12-16.5 years. A third key NREM frequency is sigma (12-15 Hz) whose power is largely determined by sleep spindles. It has previously been shown that the frequency of peak sigma

power shifts during adolescence. But the trajectory of this shift has not been established. Our longitudinal study now enables us to describe this age trend.
Semi-annually all night EEG was recorded in 92 children in 3 cohorts: C6 (n=25, 11 girls) started at age 6, studied for 4 years; C9 (n=30, 15 girls) started at age 9, studied for 7 years; C12 (n=37, 18 girls) started at age 12, studied for 6 years. EEG was recorded on consecutive nights at the subjects' homes on habitual school-night sleep schedule. Artifact-free epochs were analyzed with FFT (PASS PLUS, St. Louis). We calculated average power across the first 5 hours of NREM sleep for 0.2 Hz bands between 10.8 and 15.0 Hz, and determined the 0.2 Hz band in which sigma power showed a clear peak.
The maturational trend for sigma differed from the patterns seen for delta and theta. Maturational trends differed among frequencies within sigma: power decreased with age in low sigma frequencies (e.g. 12.0-12.2 Hz, $F_{1,861}$=64.3, $p<0.0001$) and increased with age in mid-sigma frequencies (e.g. 13.6-13.8 Hz, $F_{1,861}$=28.0, $p<0.0001$). This difference in maturational trajectories among frequencies was largely due to the steady linear increase in the frequency of peak sigma power ($F_{1,841}$=119, $p<0.0001$) with age. The rate of increase had not slowed by age 18 years.
The linear increase in frequency of peak sigma power across ages 6-18 yrs may be a manifestation of a general developmental trend to faster EEG frequencies perhaps reflecting maturation of thalamocortical circuits thought to generate sleep spindles. It is striking that the linear age trend in peak sigma frequency differs from the non-linear delta and theta maturational trends, trends that we have proposed reflect cortical maturation driven by synaptic pruning. It is biologically interesting that the age pattern of sigma differs from that of delta even though their occurrence is systematically related within NREM sleep. The remarkably robust and strikingly different maturational trajectories in the major NREM frequency bands further demonstrate the value of the sleep EEG as an arena to study, non-invasively, the neuroscience of sleep and brain development.

Disclosures: N. Darchia: None. **I.G. Campbell:** None. **I. Feinberg:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.10/HHH39

Topic: E.08. Biological Rhythms and Sleep

Title: Estimation of the number of internal states in the brain as an indicator for the conscious level and content: An ECoG study in monkeys

Authors: *T. YANAGAWA[1], N. OOSUGI[1,2], N. HASEGAWA[1], N. FUJII[1];
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Abstract: One key characteristic of consciousness is segregation and integration of psychological processes (Tononi 2004), which is believed to correlate with the network complexity of underlying brain dynamics. Previous studies showed that neural activity evoked by external stimuli had more complex pattern in conscious state than in loss of consciousness (Massimini et al, 2005). However the way to quantify the network complexity from ongoing neural activity without external stimuli was not well established. Here, our goal was to use the total number of internal states in the brain as an indicator for the network complexity, and verify its link to the level and/or the content of consciousness.
We recorded electrocoticographic (ECoG) signals from most of the lateral cortex in five macaques during awake (eye-open, eye-closed), sleeping (slow-wave), and anesthetic conditions. Ketamine-medetomidine and propofol were used for the anesthetic agents. ECoG signals were binned by 200ms non-overlapped windows, and the power spectrum was calculated for each window and each channel from 5 to 100Hz. For each frequency band, the number of internal states was defined as the number of spatial patterns in the powers, which was automatically determined by a cluster analysis. We found the number of internal states in gamma frequency band was significantly greater in the awake condition than in the anesthetic and sleeping conditions. The findings suggest that non-conscious state (under anesthesia or slow-wave sleep), compared to the conscious state (awake), could be characterized by the decrease of complexity in neural dynamics. Furthermore, the number of internal states was calculated for each cortical region. We found a significant difference between the numbers of internal states in the primary visual cortex between eye-open and eye-closed states in the awake condition, which suggests that the number of internal states could also reveal the content of consciousness (visual awareness).

Disclosures: T. Yanagawa: None. **N. Oosugi:** None. **N. Hasegawa:** None. **N. Fujii:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.11/HHH40

Topic: E.08. Biological Rhythms and Sleep

Support: Project NC 123340.1 from the Instituto Nacional de Psiquiatria Ramon de la Fuente

Grant 129103 from CONACyT to OPG

Title: Facial muscle contractions during REM sleep and its association to Rapid Eye Movements and emotional dreamed content

Authors: *A. P. RIVERA[1], I. RAMÍREZ SALADO[1], E. LÓPEZ RUIZ[1], O. PROSPÉRO-GARCÍA[2];
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Abstract: Facial muscle contractions (FMC) are features commonly associated with emotional expression during waking, yet poorly studied during sleep. Recent studies show a pattern during sleep in both healthy subjects and patients with major depression, in which FMC have a significantly higher frequency and amplitude during Rapid Eye Movement (REM) sleep than during non-REM (NREM) sleep and show an association to the Rapid Eye Movements (REMs) proper of this sleep stage. Notably, as previous studies demonstrated, REMs are also associated with emotional dream mentation (EDM). Yet, the possible functional relationship between FMC and EDM, remains unexplored.
This study analyzed FMC of the corrugator and zygomatic major -two facial muscles typically associated with emotional expression- and explores possible temporal correlations of EDM in healthy subjects during REM sleep. Additionally, it examined the interaction between FMC and REMs with EDM.
Two 8h sleep recordings were obtained from 6 female volunteers. Facial EMG recordings were obtained from the corrugator supercilii and zygomatic major (left and right) muscles. Sleep was scored using the standard AASM criteria. On the second night, FMC were visually measured.
Experimental awakenings exploring EDM (through narration, rating of a dream scale, and Dreams Qualified Report) were performed during REM sleep stages that lasted at least three minutes. Awakenings were determined by a FMC that lasted more than 100 ms and by the amplitude of any facial muscle that exceeded by 500% the background EMG activity. Additionally, experimental awakenings were performed during NREM and REM sleep stages without FMC. Following sleep recordings, FMC and REMs were quantified and analyzed for possible correlations between them. EDM global scores were gauged by exploring their correlation coefficients.
Periods with FMC and REMs were associated to higher levels of EDM in healthy subjects as compared to periods without FMC. Moreover, EDM modality (e.g. happy vs. anxious) was linked to certain muscle activation (e.g. higher FMC of zygomatic vs. lower corrugator).
The present study shows that during REM sleep with FMC (vs. periods without FMC) the corrugator, zygomatic muscles and REMs are associated to EDM. Additionally, FMC were differentially associated to emotional modality according to the activated facial

muscle.Altogether, these findings are consistent with theoretical perspectives of higher emotional variations during REM sleep associated to dream content.

Disclosures: A.P. Rivera: None. **I. Ramírez Salado:** None. **E. López Ruiz:** None. **O. Prospéro-García:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.12/HHH41

Topic: E.08. Biological Rhythms and Sleep

Support: NSF GRFP

Chateaubriand Fellowship

Title: Thalamocortical localization of human K-complexes using SEEG

Authors: *R. A. MAK-MCCULLY[1], B. ROSEN[1], H. BATUJI[3], R. CARRON[4], D. SCAVARDA[5], P. CHAUVEL[5], F. BARTOLOMEI[6], M. REY[7], E. HALGREN[2]; [2]Radiology, [1]UCSD MMIL, La Jolla, CA; [3]INSERM U879, Bron, France; [4]Service de Neurochirurgie Fonctionnelle et Stéréotaxique, Assistance Publique des Hôpitaux de Marseille, Marseille, France; [5]Faculté de Médecine, Aix-Marseille Univ., Marseille, France; [6]Lab. de Neurophysiologie et Neuropsychologie, INSERM, U751, Marseille, France; [7]Service de Neurophysiologie Clinique, Ctr. Hospitalier Universitaire Timone, Marseille, France

Abstract: K-complexes (KCs) occur as isolated downstates during stage 2 NREM sleep. KCs show a prominent frontal-central distribution in humans when measured with EEG, ECOG, or MEG. Bipolar SEEG recordings, however, are insensitive to volume conduction, allowing a highly local and focal measure of KC generation. We examined the cortical distribution of KCs and the correlation between the thalamus and cortex during KCs using SEEG recordings from patients undergoing evaluation for epilepsy. All patients studied (four male and two female, not analyzed by sex) had depth electrodes in both the thalamus and cortex. Cortical KCs were identified on a scalp EEG channel or relevant cortical bipolar contact when scalp EEG was unavailable. A significant drop in high gamma power at the time of the KC in cortical bipolar contacts verified that these events were isolated downstates (i.e. KCs). We found that KCs occur focally and locally across the cortex but are not seen in all leads. KCs can correlate with KCs in other cortical locations, as well as in the thalamus. The relative cortical and thalamic electrode locations influence these correlations. Along with KCs, spindles are the other hallmark of stage 2

sleep and have a known thalamocortical mechanism. The KC is also identical to the downstate of the slow oscillation (~1Hz) that emerges in stage 3 sleep. The slow oscillation has been considered a cortico-cortico process that may be synchronized by the thalamus; therefore, understanding the thalamocortical interactions underlying KCs helps elucidate its relationship to other sleep graphoelements, as well as its function.

Disclosures: R.A. Mak-Mccully: None. **B. Rosen:** None. **R. Carron:** None. **D. Scavarda:** None. **H. Batuji:** None. **P. Chauvel:** None. **F. Bartolomei:** None. **M. Rey:** None. **E. Halgren:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.13/HHH42

Topic: E.08. Biological Rhythms and Sleep

Support: NIH

Title: Occurrence of delta rhythm in awake human intracranial local field potential recordings

Authors: *R. N. SACHDEV[1], I. I. GONCHAROVA[2], N. GASPARD[2], L. J. HIRSCH[2], D. A. MCCORMICK[3], D. D. SPENCER[4], H. P. ZAVERI[2];
[1]Neurobio., Yale Univ. Sch. of Med., NEW HAVEN, CT; [2]Neurol., [3]Neurobio., [4]Neurosurg., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The scalp EEG and the intracranial local field potential of the neocortex are widely used as a measure of the cortical state. At any one moment during sleep the entire cortex is thought to be in a single state, i.e. in slow wave or rapid eye movement sleep. Similarly, during wakefulness the entire cortex is thought to be in a persistently activated state where the EEG is dominated by frequencies in the theta to gamma range (5 Hz and upwards). Recent work in rodents has challenged these ideas about the nature of neocortical activity during wakefulness. In quietly sitting awake mice the local field potential and membrane potential of single neurons in somatosensory and auditory cortex can show oscillations in the delta (1-4 Hz) range. Whether local delta is simply a phenomenon of rodent neocortex or also occurs in the human brain is not known. Here we used chronic (up to 2 week-long) intracranial EEG recordings in patients, who were being prepared for surgical intervention, to look for evidence of local slowing during wakefulness. Two one hour-long segments of video and intracranial EEG, were taken for analysis. One epoch was 2-3 days after implant of > 100 intracranial electrode contacts that spanned a large and variable extent of the neocortex, and a second epoch obtained from the same

electrodes occurred ~ 7-8 days after the electrodes had been implanted, and anti-epileptic drugs had been tapered off or stopped. Video for each hour was inspected for whether the patients were active and awake. These recordings reveal that in all 12 patients, before and after drugs had been tapered off, there was significant (> 3 standard deviations of the mean delta power estimated over an average of 114 contacts) delta activity on approximately 10 % of the recording sites, even when other recording sites showed little evidence of delta activity. The cortical area that showed delta rhythm varied from patient to patient, and the site was distinct from the post-operatively determined site of seizure onset. During the epochs of delta, local recording sites (within a centimeter) showed significant coherence, while more distant recording sites showed no significant coherence, indicating that this state was not akin to the epochs of slow wave sleep, when the entire montage of > 100 recording sites show very similar, coherent delta rhythm. Thus, during wakefulness even in human beings, there is no obligatory, spatially broad, persistently activated state dominated by high frequency activity. Instead, when people are awake some cortical areas can be in states dominated by delta rhythm.

Disclosures: R.N. Sachdev: None. **I.I. Goncharova:** None. **N. Gaspard:** None. **L.J. Hirsch:** None. **D.D. Spencer:** None. **H.P. Zaveri:** None. **D.A. McCormick:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.14/HHH43

Topic: E.08. Biological Rhythms and Sleep

Support: NSU PFRDG

Title: The effect of chronotype on stress response, sustained attention, and emotional memory

Authors: C. GOBIN[1], A. I. FINS[2], J. B. BANKS[1], *J. L. TARTAR[1];
[1]Farquhar SBS, [2]Ctr. for Psychological Studies, Nova Southeastern Univ., DAVIE, FL

Abstract: Extreme morning or evening chronotypes often experience difficulties in emotion processing and report mood disturbances. For example, individuals with an extreme evening preference have a high prevalence of learning problems and poor psychological health (Dagan and Einstein, 1999; Ottoni, Antoniolli & Lara, 2012; Shirayama et al., 2003) and individuals with an extreme morning preference show higher rates of depressive disorders (Xu, et al., 2005). However, the extent to which chronotype affects psychological functioning amidst stress is unknown. In an effort to shed light on this uncertainty, we examined the relationship between chronotype and stress responsivity. We administered a placebo or experimental cold-pressor test

(CPT) stressor prior to the cognitive and emotional task in the early morning (0900-1000) or evening (2100-2200) at times that were both congruent and incongruent with participant's self-reported chronotype in an independent samples design. Following placebo or stress exposure, participants were tested on emotional memory recognition and sustained attention (SART task) performance. In addition to the chronotype scale (MEQ), participants also filled out a series of questionnaires related to psychological health (CES-D, STAI, and POMS) and a sleep quality inventory (PSQI). Cortisol levels before and after the stressors were also measured. We also tested how chronotype-associated polymorphisms in the Per3 gene are related to the ability of acute stress to affect emotional and non-emotional task performance in the morning and evening. Preliminary analyses indicate that performance for an emotional task is affected when the testing time is incongruent to an individual's chronotype. However, this is not the case for a non-emotional sustained attention task- testing time does not affect SART task performance at chronotype-incongruent times. Combined these findings illustrate how individual chronotype can affect cognition and stress responses.

Disclosures: C. Gobin: None. **A.I. Fins:** None. **J.L. Tartar:** None. **J.B. Banks:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.15/HHH44

Topic: E.08. Biological Rhythms and Sleep

Support: CIRUS Postdoctoral Award, NHMRC

Title: Computational study of sleepiness and circadian rhythms on rotating shift schedules

Authors: *S. POSTNOVA[1], D. D. POSTNOV[2], P. A. ROBINSON[1];
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Abstract: Desynchrony of circadian rhythms and disturbed sleep during shiftwork lead to fatigue and significantly increased risks of cancer, diabetes, and heart disease. Nevertheless, shiftwork is an integral part of our 24-h society, and an average of 20% of the population work shifts. It is, thus, essential to find conditions reducing sleepiness and circadian desynchrony in shiftworkers. However, experimental studies are highly mixed due to the enormous number of different schedules used in industries. Here we use computational approach to examine sleep and circadian dynamics on widely spread 3-shift rotating systems in order to pin down the biological mechanisms underlying experimental observations and to predict shift schedules with minimal disturbances.

We use a physiologically based mathematical model of sleep-wake cycles and circadian system which incorporates the mutual inhibition between the sleep-active ventrolateral preoptic nucleus of the hypothalamus and the wake-active monoaminergic nuclei in the brainstem and hypothalamus. The switch in the dynamics of these nuclei is controlled by the homeostatic and circadian processes, with the circadian process being regulated by the suprachiasmatic nuclei of the hypothalamus. The circadian phase is entrained by light input, which is itself changed by shift times and sleep dynamics. This combined model has been validated on a number of sleep phenomena, including normal sleep, circadian desynchrony, and permanent shiftwork.
Using this model we examined how sleep and circadian dynamics are affected by three major factors: (i) rotation speed, i.e., number of days on each of the 3 shift types; (ii) shift start time; and (iii) direction of rotation, i.e., forward versus backward. Consistent with experimental observations we find that for forward schedules very quick and very slow rotation leads to significantly lower sleepiness compared to intermediate rotation speeds. In this case shift start time does not have a significant effect on the resulting sleepiness. The situation is different for backward rotation. In this case shifts starting around 04:00 - 05:00 have low sleepiness even at intermediate rotation speeds, while quick rotation can be associated with significantly higher sleepiness depending on the duration of changeover time between the different shift types. We also find that forward rotation is generally better than backward for quick (1-2 days) and slow (>9 days) rotation, but backward is of advantage for intermediate speeds. This study helps explaining mixed experimental observations, and provides a framework for better understanding of sleep dynamics during shiftwork.

Disclosures: S. Postnova: None. **D.D. Postnov:** None. **P.A. Robinson:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.16/HHH45

Topic: E.08. Biological Rhythms and Sleep

Support: DFG MO930/4-1

Lichtenberg Grant 86 507

Title: Single units in the human medial temporal lobe during propofol anesthesia

Authors: *J. NIEDIEK[1], M. NAVRATIL[1], V. A. COENEN[2], C. E. ELGER[1], M. SOEHLE[3], F. MORMANN[1];

[1]Dept. of Epileptology, [2]Stereotaxy and MR based OR Techniques, Dept. of Neurosurg., [3]Dept. of Anaesthesiology and Intensive Care Med., Univ. of Bonn, Bonn, Germany

Abstract: General anesthesia induced with the anesthetic drug propofol globally reduces neuronal activity in a dose-dependent manner. However, the effect of propofol administration on single neurons in the human brain cannot be examined with classical non-invasive methods such as fMRI, PET, or EEG. We used single-unit recordings from, up to now, eight epilepsy patients to investigate the precise time-course of single neuron activity in the human medial temporal lobe (MTL) during propofol anesthesia.
We recorded the activity of single neurons in the MTL of neurosurgical patients undergoing epilepsy monitoring prior to resective surgery. These patients were implanted with, most often, eight intracerebral depth electrodes to precisely locate the epileptic focus. Each depth electrode was equipped with eight micro-wires protruding from its tip. The electrodes were explanted after the epilepsy monitoring had ended, typically one or two weeks after implantation. For the anesthesia protocol, estimated propofol effect-site concentrations were raised to a level where loss of consciousness occurred, and then further until burst suppression was observed.
We analyzed the activity of 38 single- and multi-units in the hippocampus, amygdala, entorhinal cortex, and parahippocampal cortex. All units completely ceased to fire at high propofol concentrations, and started to fire again when the propofol concentration dropped. All observed units maintained their baseline firing rate over an extended range of low propofol concentrations. Remarkably, when the propofol concentration reached a certain critical level, units stopped firing in an abrupt, rather than gradual, manner. Hippocampal neurons showed a tendency to stop firing earlier than did neurons in other MTL regions. Furthermore, the clinical loss of consciousness appeared to coincide with the cessation of neuronal activity in the MTL.
Our data reveal that during propofol anesthesia, neurons in the human MTL maintain their firing across a large range of propofol concentrations, but completely stop firing when a critical concentration is reached. Behavioral studies have shown that propofol administration reduces performance in memory tasks associated with the MTL. Whether the observed firing cessation is a neural correlate of impaired memory function remains to be tested in future studies.

Disclosures: J. Niediek: None. **M. Navratil:** None. **V.A. Coenen:** None. **C.E. Elger:** None. **M. Soehle:** None. **F. Mormann:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.17/HHH46

Topic: E.08. Biological Rhythms and Sleep

Title: Do specific common sleep postures independently evoke headaches and breathing obstruction?

Authors: N. AL-TIMIMI[1], *J. GILLICK[2];
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Abstract: Specific common twisted sleep postures are independently associated with: 1) onset, recurrence and laterality of pattern headache disorders (pHA); and, 2) the presence of sleep disorders.
Data:
A history and myofascial-exam data sheet was completed during a comprehensive general medical evaluation in 367 consenting compensation applicants. The data sheet was attached to exam-notes and a copy given to the subject. After limited data extraction, the report and notes were submitted/ filed. GpA, the first 109, selected for TMJ, bruxism or jaw tenderness, were queried in specifics of sleep posture, HA details, and work impact. GpB, 258 unselected, sequential subjects provide prevalence and parallel, less detailed data.
Current Sleep Postures: The evaluator and each subject mutually agreed on the accuracy of sleep posture recorded:
TCU: (GpB=201/258; GpA=106/109) (Twisted ± curled-up) as current 1° sleep posture ⇒ chest side-twisted down, face-neck twisted to breathe, Knee(s) bent >90°; and,
NBP: (GpB=37; GpA=0) (Neutral body posture) as current 1° sleep posture ⇒chest, neck, face are neutral, untwisted; knees bent <90°.
Pattern headaches (pHA) are similar in: onset time; duration; start area; impairment level; frequency; ± autonomic signs.
Results:
Pattern headaches (pHA)-GpB -(also reflect GpA)
pHA frequency in 201 TCU is 90% = 30-x's > 3% in 37 NBP.
Face-neck twist direction & pHA side: both ⇒right in 61%.
Face-twist direction & pHA side, both ⇒left in 38%.
Neck pain (CS) in TCUs is 90% = 8-x's > 16% in NBP.
pHAs are 83% ipsilateral with CS: right ⇒56%; left ⇒27%; mixed ⇒17%
Pterygoid (LPt) muscle spasm with Trigeminal ganglion hypersensitivity :TCU is 96% = 32-x's > 3% in NBP.
LPt, pHA, & CS =79% ipsilateral dominant: right ⇒56%; left ⇒23%; mixed ⇒21%
pHA- Functional impact details (GpA):
Affect: 86% (91) have pHA: 1/3-ignore it as background; 1/3 episodically self-treat; &, 1/3 affect ADL's enough to seek medical advice ± self-treat.
pHA- Work Loss (>6 hr/wk) occurs in 63%= 67 [46% ⇒lose focus ≥2 hr ≥3d/wk; 16% ⇒non-productive or absent ≥5 x ½d/mo; 38%⇒lose 2 to 6 full days/wk.]

Sleep Dysfunctions (SD) are common in TCU:
They include: Proven sleep apnea (OSAS); Suspected sleep apnea- history alone (sOSA); & Pain, psych and snoring (Other).
GpA: In the 106 with TCU, SD= 93%: OSAS=46%; sOSA= 25%; & Other= 27%.
GpA has no NBPs.
GpB: In the 201 with TCU: SD= 95%= 12-x's >8% in NBP; OSAS= 48%, 0 in NBP; sOSA= 33%, 0 in NBP; &, Other= 16%= 2-x's >8% in NBP
Start of TCU behavior,
TCU, ±32%, start as small children; 68% start as adults⋯including related background presence of pHAs, neck stiffness, restless nights, etc.
Data details will be posted at www.PainDataMine.com

Disclosures: N. Al-timimi: None. **J. Gillick:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.18/III1

Topic: E.08. Biological Rhythms and Sleep

Support: NWO 452-08-013

Title: Sleep slow oscillations modulate brain-wide information processing

Authors: *R. COX, J. VAN DRIEL, L. M. TALAMINI;
Dept. of Psychology, Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: During deep sleep, slow rhythmic brain signals alternate between depolarized UP states and hyperpolarized DOWN states at a frequency around 0.7 Hz. These slow oscillations travel in a predominantly anterior-to-posterior direction across the scalp. UP states are characterized by greater sleep spindle (10-16 Hz) and gamma (50-80 Hz) power, higher neuromodulatory activity, enhanced excitability and increased spiking. Thus, most brain processing appears to occur in the UP, rather than the DOWN state. Although studies of functional connectivity across various sleep stages have been performed, the question of whether and how slow oscillations modulate functional connectivity remains unexplored.
We detected slow oscillation peaks and troughs in 128-channel EEG recordings of six napping participants, using multiple seed electrodes located at frontal, central and parietal midline sites as well as left- and right-sided sites. Resulting UP and DOWN state epochs were compared in the time-frequency domain between 3 and 100 Hz across all recording sites. We investigated power

modulations, phase synchrony and cross-frequency coupling, both within and between sites. Preliminary data reveals a strong coupling of power in the sleep spindle and gamma bands to the UP state. Spatially, this coupling was largely limited to a topographical region surrounding the seed electrode used for UP/DOWN state detection. Combined with existing evidence of slow oscillations behaving as 'traveling waves', our data suggest that as UP states move across the scalp, they carry with them an island of enhanced information processing. Ongoing analyses will provide additional insight into how measures of functional connectivity are affected by slow oscillations.

Disclosures: R. Cox: None. **J. van Driel:** None. **L.M. Talamini:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.19/III2

Topic: E.08. Biological Rhythms and Sleep

Title: Cumulative sleep restriction alters lipid metabolism - transcriptomic and metabolomic studies in humans

Authors: *V. AHO[1], H. M. OLLILA[2], T. PORKKA-HEISKANEN[3];
[1]Inst. of Biomedicine, Univ. of Helsinki, Helsinki, Finland; [2]Natl. Inst. for Hlth. and Welfare, Helsinki, Finland; [3]Inst. of Biomedicine, Helsinki, Finland

Abstract: Objectives: Short or insufficient sleep has been associated with increased risk for metabolic diseases, such as atherosclerosis and type II diabetes. To elucidate the molecular mechanisms linking sleep homeostasis and metabolism, we compared transcriptomic and metabolomic profiles of partially sleep-deprived subjects to normally sleeping controls at experimental and epidemiological levels.
Materials: For the experimental study simulating a 5-day working week with restricted sleep, subjects spent a week in laboratory conditions. After two nights' baseline sleep (8 hours sleep/night), subjects were partially sleep deprived for five successive nights (4 h/night) followed by two nights' recovery sleep (8 h/night). Control subjects spent the time in laboratory sleeping normally (8 h/night). Fasting blood samples were collected from 10 sleep-deprived and 6 control subjects after baseline, sleep restriction (SR), and recovery phases.
The epidemiological sample was a subset of a Finnish population-based sample Finrisk2007. For the DILGOM subsample, subjects (N=518) filled a questionnaire including questions on sleep sufficiency and gave fasting blood samples.

Methods: Lipid profiles including lipoprotein particle species, cholesterol, and triglycerides were measured from serum with NMR metabolomics. Total RNA was extracted from blood mononuclear leukocytes, and RNA expression was analysed using Affymetrix whole genome microarrays complemented with pathway analyses of differentially expressed genes.
Results: The most enriched pathways among genes that were down-regulated after experimental SR were involved in metabolic processes, such as cholesterol transport and homeostasis ($P<0.001$). Supporting this, in the epidemiological sample, these pathways were also enriched among genes whose expression was lower in subjects reporting insufficient sleep (SRIS) ($P<0.05$).
However, the metabolomic screening showed a different effect for the two samples. In the experimental study, the lipoprotein profile after SR seemed beneficial with less low density lipoprotein (LDL) particles ($P<0.01$), apolipoprotein B (apoB) ($P<0.005$), and LDL cholesterol ($P<0.0005$). On the contrary, in the epidemiological sample the SRIS had lower levels of high density lipoproteins (HDL) ($P<0.01$).
Conclusions: Under conditions of both short-term and long-term sleep restriction, lipid - particularly cholesterol- metabolic pathways are significantly modified at the level of transcription as well as at the level of serum lipoprotein profiles. We propose that these mechanisms may contribute to the development of cardiovascular diseases.

Disclosures: V. Aho: None. **H.M. Ollila:** None. **T. Porkka-Heiskanen:** None.

Poster

570. **Sleep Systems: Humans, Monkeys, and Models**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 570.20/III3

Topic: E.08. Biological Rhythms and Sleep

Support: NR013693

Title: Global brain blood-oxygenation level dependent responses to autonomic challenges in obstructive sleep apnea

Authors: *P. M. MACEY[1,2], R. KUMAR[3], J. A. OGREN[1], M. A. WOO[1], F. L. YAN-GO[4], R. M. HARPER[3];
[1]UCLA Sch. of Nursing, [2]Brain Res. Inst., [3]Dept. of Neurobio., [4]Dept. of Neurol., Univ. of California at Los Angeles, Los Angeles, CA

Abstract: Obstructive sleep apnea (OSA) is accompanied by brain injury and dysfunction in regions that regulate autonomic action, which may impair regulation of cerebral blood flow and

oxygenation and contribute to other syndrome pathologies, such as exaggerated sympathetic tone. The objective was to assess cerebral blood oxygenation responses to autonomic challenges in OSA over control subjects. We separately assessed female and male patterns, since OSA characteristics and brain injury differ between sexes. We studied 94 subjects, including newly-diagnosed, untreated moderate-severe OSA patients (6 female age mean±std: 52.1±8.1 years; 31 male 54.3±8.4 years), and healthy control subjects (20 female age 50.5±8.1 years; 37 male age 45.6±9.2 years). We measured responses every 2 s with the global blood-oxygenation level dependent (BOLD) signal using an MRI scanner while subjects performed cold pressor, hand grip, and Valsalva maneuver challenges. All challenges elicited rapid changes in BOLD signals which differed between groups to the cold and hand grip challenges (cold pressor peak at 8 s: OSA 0.14% vs. 0.31% in controls; hand grip: peak at 6 s in control, but decline in OSA), but not to the Valsalva (repeated-measures ANOVA, $p<0.05$). OSA females showed greater differences in magnitude and temporal patterns of responses relative to their healthy counterparts from the OSA males (cold pressor: female OSA peak 0.19% at 8 s vs. 0.35% at 12 s in controls, 8 s peak male OSA 0.13% vs. 0.34% in control; hand grip: no female OSA vs. control differences, 6 s peak male OSA 0.04% vs. 0.30% in controls; Valsalva: at 4 s 1% lower in female OSA vs. control, no difference in males). Obstructive sleep apnea patients show reduced and slower-to-develop brain BOLD responses to autonomic challenges, relative to healthy controls. The impaired brain and peripheral vascular responses are more pronounced in females than males. The findings emphasize the consequences of OSA to vascular processes, which have the potential to result in further neural injury, interfere with timely perfusion of brain tissue, and modify performance of those structures.

Disclosures: P.M. Macey: None. **R. Kumar:** None. **J.A. Ogren:** None. **M.A. Woo:** None. **F.L. Yan-Go:** None. **R.M. Harper:** None.

571**Poster**

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.01/III4

Topic: F.01. Human Cognition and Behavior

Support: ERC Grant Agreement no 249640

Title: Multi-voxel pattern analyses reveal similarities and differences in the semantic representations of words and objects

Authors: B. J. DEVEREUX, A. CLARKE, A. MAROUCHOS, *L. K. TYLER;
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Abstract: Understanding the meaning of words and objects (e.g. “apple”) requires the activation of underlying conceptual representations. Such representations are assumed to be coded such that meaning can be evoked irrespective of the stimulus modality, a view which is supported by evidence of a common semantic system that is activated for both words and objects (e.g. Bright et al. 2004; Martin 2007). However, activation of a common semantic system does not necessitate that these regions represent the same information during word and object processing. A stronger test for modality-invariant semantic representations is provided by multi-voxel pattern analysis (MVPA), which, further to identifying regions involved for both words and objects, also allows us to characterize the similarity of information content in those regions across modality. We ask whether representations are similar across modalities in an fMRI study where words and pictures representing 60 concepts were presented. For both modalities, participants responded with a category-level name for each item. Activation maps for each word and object were extracted and used as input to two searchlight-based MVPA techniques. The first method was representational similarity analyses (RSA). In this model-driven approach, three representational dissimilarity matrices (RDMs) were computed, which captured low-level visual properties of the words and objects and the semantic category structure common to both. These were then correlated with searchlight activation RDMs. The second analyses was a novel data-driven method, using cluster analysis to identify regions of shared information content across modalities.
The RSA revealed strong correlations between the visual model RDMs and the dissimilarity patterns in early visual cortex for both words and objects. For the semantic RDM, there was an extensive left-lateralized network of significant correlations for the object data, including the left fusiform gyrus, lateral occipital complex, left posterior middle temporal gyrus (LpMTG) and left angular gyrus (LAngG). The word data showed significant correlations more anteriorly into left MTG, but no significant correlations in the fusiform. As with the object data, there were also significant correlations in LpMTG and LAngG, suggesting these regions may form part of a core semantic network activated during semantic processing of both words and objects. However, the data-driven cluster analysis method showed that the representations computed in these regions do not cluster together across modality, suggesting these regions may play different functional roles for the semantic processing of words and objects.

Disclosures: B.J. Devereux: None. **L.K. Tyler:** None. **A. Clarke:** None. **A. Marouchos:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.02/III5

Topic: F.01. Human Cognition and Behavior

Support: NIDCD Grant K24-DC010028

NINDS Grant NS040596

Title: Multiple time scales of auditory stimulus adaptation in human cortex

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Abstract: It is well established that cortical auditory evoked responses decrease with stimulus repetition in animals and humans, a phenomenon known as stimulus-specific adaptation (SSA). Little is known, however, about the effects of SSA on high-frequency cortical oscillations (> 60 Hz) which are not phase-locked and are used increasingly to investigate human cortical processing. To address this issue, we recorded electrocorticographic (ECoG) activity from six epilepsy patients with implanted subdural electrode arrays. An auditory odd-ball paradigm was used with tones (frequent: 1000 Hz; infrequent: 1200 Hz) and speech (frequent: /ba/; infrequent: /da/), as well as reversed and equal stimulus probability conditions. Time-frequency analysis was performed to measure statistically significant event-related changes in high gamma (70-150 Hz) power relative to pre-stimulus baseline. Time-domain averaging was used to derive concurrent auditory evoked responses (N100). We found strong adaptation of high gamma power at electrode sites where auditory responses were elicited. Responses to infrequent stimuli were stronger than responses to frequently repeated stimuli for both tones and speech, consistent with previous SSA studies. Multiple adaptation time scales were identified, including rapid adaptation at the onset of a stimulus block and slower adaptation across sequences of stimulus repetitions. Infrequent stimuli and equal-probability stimuli elicited similar responses, suggesting that differences in responses to high and low probability stimuli reflected adaptation of the high probability response. Although the auditory evoked N100 showed the expected pattern of adaptation, the relationship between high gamma and evoked response adaptation was variable. These results suggest a mechanism for coding high probability auditory inputs that adapts to stimulus regularities on multiple time scales.

Disclosures: S. Eliades: None. **N.E. Crone:** None. **D. Boatman-Reich:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.03/III6

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01 NS065049

Title: Effect of visual context on the activation of move- and use-related actions during semantic object processing

Authors: *A. D. SHAPIRO[1], S. KALÉNINE[2], A. FLUMINI[3], A. M. BORGHI[3,4], L. J. BUXBAUM[1];
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Abstract: A number of lines of evidence suggest that observation of manipulable objects activates the motor system (e.g., Chao & Martin, 2000; Craighero, Bello, Fadiga, & Rizzolatti, 2002; Martin, 2007; Noppeney, 2008; Tucker & Ellis, 1998). Many manipulable objects, however, are associated with several actions. For example, a tape dispenser may be grasped with a power grip (clench) to move it, whereas the end of the tape may be grasped with a precision grip (pinch) to use it. Recent studies have shown that object processing may recruit both of these action types (Bub, Masson, & Cree, 2008), and that they may compete with each other within single objects (Jax & Buxbaum, 2010; Jax & Buxbaum, 2013). This study explored the hypothesis that evocation of move- or use-related actions is responsive to the congruence of the visual scene in which objects are presented.
Twenty-one healthy adults were asked to categorize object pictures presented in different naturalistic visual contexts that evoke either move- or use-related actions (e.g. tape dispenser in drawer vs. on desk top). Categorization judgments (natural vs. artifact) were performed by making a move- or a use-related action (clench vs. a pinch) on a response device. Categorization reaction times were analyzed as a function of Context (use or move) and Gesture (clench or pinch). Although the actions performed were irrelevant to the categorization judgment, responses were significantly faster when actions were compatible (use context-pinch, move context-clench) compared to incompatible (use context-clench, move context-pinch) with the visual context. Prior demonstrations indicate that action evocation during object processing may be modulated by action intentions (e.g, Pavese & Buxbaum, 2002), distance from the viewer (e.g., Costantini, Ambrosini, Scorolli, & Borghi, 2011), relationships to other objects (Borghi, Flumini, Natraj, & Wheaton, 2012) and verbal context (e.g., Lee, Middleton, Mirman, Kalénine, & Buxbaum, 2013). These data extend such findings by demonstrating that activation of move and use-related gestures during semantic object processing may additionally be modulated by the visual scene in which the objects are presented, with clear implications for object processing in naturalistic tasks.

Disclosures: A.D. Shapiro: None. **S. Kalénine:** None. **A. Flumini:** None. **A.M. Borghi:** None. **L.J. Buxbaum:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.04/III7

Topic: F.01. Human Cognition and Behavior

Title: Contrasting CMSs activity during core-self and autobiographical-self processes

Authors: *H. F. ARAUJO[1,2], J. KAPLAN[1], H. DAMASIO[1], A. DAMASIO[1];
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Abstract: At any moment, individuals may access information that pertains to facts and events of their biographies - "the autobiographical self" (Damasio 1998) or "narrative self" (Gallagher 1999) - as well as information that pertains to their current body states - "the core self" (Damasio 1998). The neural bases of these two kinds conscious self processes remain uncertain, although a frequent account associates activity in cortical midline structures (CMSs) with self-reference (Northoff et al. 2006). Here, we investigate the involvement of CMS in domains of autobiographical self and in domains of core-self.
We conducted a block-design fMRI study, in which 19 subjects answered questions about their own traits, their general biography, their current interoceptive states and their current exteroceptive states (4 experimental conditions). In each run, each of the conditions (blocks of 24 seconds) was repeated 3 times and separated by a "one-back task" (also in blocks of 24 seconds). The one-back-task was used as a baseline in order to ensure disengagement of self-related processing. There were three runs per study.
Preliminary analysis of data shows that, compared with conditions of core-self, conditions of autobiographical self are associated with greater activity in CMSs (namely medial prefrontal, anterior cingulate and posteromedial cortices). The reverse contrast (core-self > autobiographical self) yields greater activity in the posteromedial cortex (PMC). These results suggest that the involvement of CMSs in the assessment of self-related information varies according to the kind and quality of the representations involved.
References
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Northoff, G. et al., 2006. Self-referential processing in our brain_A meta-analysis of imaging studies on the self. NeuroImage, 31(1), pp.440-457.

Disclosures: H.F. Araujo: None. **J. Kaplan:** None. **H. Damasio:** None. **A. Damasio:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.05/III8

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant NS21135

Nielsen Corporation

Title: Neural responses to musical consonance and dissonance in the human superior temporal gyrus

Authors: *F. FOO[1], D. KING-STEPHENS[2], P. B. WEBER[2], K. D. LAXER[2], R. T. KNIGHT[1]; [1]Univ. of California, Berkeley, CA; [2]Neurol. and Neurosurg., California Pacific Med. Ctr., San Francisco, CA

Abstract: Research on the neural basis of consonance and dissonance processing in music has depended on non-invasive electrophysiological and functional imaging techniques in humans. However, the fine-grained spatiotemporal dynamics of consonance and dissonance perception within the auditory cortex remain unknown. Using Electrocorticography (ECoG) that records electrophysiological signals directly from subdural grids located on the lateral surface of human cortex, we aimed to investigate whether subregions of the superior temporal gyrus (STG) are differentially responsive to consonant and dissonant chords. We recorded from two patients with extensive ECoG coverage in either left or right STG and additional frontal sites, as they passively listened to highly consonant and highly dissonant piano chords. We observed two cortical sites of interest within the left STG: one that had no difference in response to both consonant and dissonant chords, and another, 2 cm anterior to the former site, that showed increased responses to dissonant chords in the high-gamma frequency range (HG; 70-150Hz) ($p<0.05$, FDR corrected) within the first 100ms after stimulus onset. Event-related potentials showed a similar spatial distribution pattern for consonant and dissonant chords in the STG. Similar HG effects were observed in the right STG, where a cortical site not differing in response to consonant and dissonant chords had an adjacent site (1 cm inferior) that showed increased HG responses to dissonant chords. These data indicate that sensitivity to consonant and dissonant

chords within the STG is apparent at a resolution of 1-2 cm in both hemispheres. Additionally, we observed that a site in left inferior frontal cortex showed increased HG activity 300ms after stimulus onset only for dissonant chords that likely represents a subsequent orienting response to the musical dissonance. We propose that the increase in HG activity for dissonant chords reflects heightened synchronous neuronal firing in response to the phenomena of beats or "roughness" that characterize our perception of dissonance. In sum, the findings indicate that direct cortical recording can track perceptual aspects of musical processing with temporal and spatial precision.

Disclosures: F. Foo: None. **D. King-Stephens:** None. **P.B. Weber:** None. **K.D. Laxer:** None. **R.T. Knight:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.06/III9

Topic: F.01. Human Cognition and Behavior

Title: Relationship between brain activity and sense of self-agency during kinesthetic illusory feeling induced by tool use: a functional near-infrared spectroscopy sdtuy

Authors: *S. WAKATA[1,2], S. MORIOKA[2];
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Abstract: [Objective]
When using a tool, the tool becomes assimilated as a part of the body, it is thought that the body schema extends dynamically in real time (Iriki,1996). Self-agency is the feeling that "the body is performing this movement by itself" (Gallagher, 2000), and can be involved when we observe movement out of the body. For example, It is involved when the movement of fingers projected onto a screen lined up with the position of a hand is passively observed (Kaneko, 2004). However, it is unclear whether self-agency is involved when the movement of a tool is projected onto a screen lined up with the position of a hand; furthermore, the underlying neural networks have yet to be elucidated. This experiment aimed to clarify these questions by using functional near-infrared spectroscopy.
[Materials & Methods]
Twelve healthy, right-handed subjects were enrolled in the study. Participants were seated on chairs, and asked to look at a screen. The illusion condition was displayed on the screen as extension of a hand, and the non-illusion condition was shifted on the screen approximately 15

cm left. The tool used was a tongue. Subjective ratings of self-agency were measured using a questionnaire. To determine the difference in the subjective ratings of self-agency between the conditions, we performed a Wilcoxon signed-rank test. To determine the difference in oxygenated hemoglobin (oxyHb) levels between the conditions, we performed one-tailed sample t-tests for each region of interest. Finally, Spearman's rank correlation coefficient was used to evaluate the relationship between oxyHb levels and the subjective ratings of the illusion. The threshold for statistical significance level was set at $p < 0.05$.
[Results]
The illusion condition was scored significantly higher than the non-illusion condition in the subjective ratings of self-agency. In the right prefrontal area, the response during the illusion condition was significantly increased compared to the response during the non-illusion condition, Furthermore, there was a positive correlation between the oxyHb increase and the subjective ratings of the illusion. In the left primary sensory-motor area, the response during the non-illusion condition was significantly increased compared to the response during the illusion condition; moreover, there was a negative correlation between the oxyHb increase in this area and the subjective ratings of the illusion.
[Conclusion]
The left primary sensory-motor area and the right prefrontal area are related to self-agency during kinesthetic illusory feeling induced by tool-use.

Disclosures: S. Wakata: None. **S. Morioka:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.07/III10

Topic: F.01. Human Cognition and Behavior

Support: European Research Council

Swedish Foundation for Strategic Research

James S. McDonnell Foundation

Swedish Research Council

Söderbergska Stiftelsen

Title: Mental imagery and multisensory integration

Authors: *C. C. BERGER, H. H. EHRSSON;
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Abstract: Multisensory interactions are the norm in perception, and an abundance of research on the interaction and integration of the senses has demonstrated the importance of combining sensory information from different modalities on our perception of the external world (Stein & Stanford, 2008). However, although research on mental imagery has revealed a great deal of functional and neuroanatomical overlap between imagery and perception (Kosslyn, Ganis, & Thompson, 2001), this line of research has primarily focused on similarities within a particular modality and has yet to address whether imagery is capable of leading to multisensory integration. In recent behavioral experiments, we found evidence in favor of multisensory integration or real and imagined stimuli in adapted versions of classic multisensory illusions. Here, we present the results of a psychophysics ventriloquism experiment (n = 18) and a functional magnetic resonance imagining experiment (fMRI) experiment (n = 22) on an adapted version of the classic ventriloquism illusion that provide convergent evidence of imagery-perception multisensory integration. In the psychophysics ventriloquism experiment, we found that the perceived location of sound became ambiguous earlier on in the spatial 'staircase' when participants imagined the appearance of a centrally located visual stimulus than when no visual stimulus was imagined [$t(17) = 2.39$, $p = 029$], an effect that can be attributed to the translocation of sound toward the imagined visual stimulus. In our fMRI experiment, we found that temporally synchronous (vs. temporally asynchronous) imagined visual stimuli lead to a significant translocation of stationary auditory stimuli towards the
imagined visual stimuli [$t(22) = 2.50$, $p = .021$] and significant activation ($t = 3.34$, $p = .032$, corrected) in the left superior temporal sulcus—a key site for the integration of audiovisual stimuli (Noesselt et al., 2007). These findings provide support for perceptually based theories of imagery and suggest that neuronal signals produced by imagined stimuli can integrate with signals generated by real stimuli of a different sensory modality to create robust multisensory percepts.

Disclosures: C.C. Berger: None. **H.H. Ehrsson:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.08/III11

Topic: F.01. Human Cognition and Behavior

Support: European Research Council

Swedish Foundation for Strategic Research

Human Frontier Science Program

James S. McDonnell Foundation

Swedish Research Council

Söderberska Stiftelsen

Title: The construction of a whole body multisensory gestalt by the human premotor cortex

Authors: *G. GENTILE, M. BJÖRNSDOTTER, V. I. PETKOVA, Z. ABDULKARIM, H. H. EHRSSON;
Karolinska Institutet, Stockholm, Sweden

Abstract: How does the brain construct a unified representation of one's own body that assembles all of its parts into a perceptually coherent whole? Previous research has shown that the integration of bodily signals in multisensory fronto-parietal areas underlies the perception of a limb or an entire body as part of one's physical self. In the present study, we sought to investigate the mechanisms that support the construction of a whole body perceptual gestalt from the representation of its individual parts. In a behavioral experiment on 20 healthy participants, we extended previous work on full-body ownership illusions and showed that the integration of visuo-tactile signals from one of 3 body parts (hand, torso or leg) leads to the generalization of the feeling of ownership from the stimulated body part to the whole body (questionnaire data; $p<0.001$). In an fMRI experiment involving 16 healthy participants, we induced the feeling of ownership of an entire body by applying synchronous touches to the hand, torso, or leg of the participant's unseen real body and a mannequin in direct view from the first person perspective. Asynchronous visuo-tactile stimulation was used as control. We employed multivariate classification to identify patterns of neural activity that were either specific to the representation of a single body part or that generalized significantly across all body parts to create a unified representation of a self-attributed whole body. First, we found that neuronal populations in the left ventral premotor cortex exhibited illusion-specific activity patterns that generalized across all 3 body parts ($p<0.05$ corrected), in line with the construction of a whole body perceptual gestalt. Crucially, this "spread of ownership" from one part to the whole was abolished in a control condition with synchronous visuo-tactile stimulation to a detached hand, proving the importance of viewing an entire body for the full-body illusion to arise. Second, we found that neuronal populations in the bilateral ventral premotor cortices and in the left intraparietal cortex integrated visuo-tactile signals in a body-part-specific fashion ($p<0.05$ corrected), giving rise to disjointed representations of the three body segments. We propose that, on one side, bodily signals are integrated in a body-part-specific fashion by neurons that possess visuo-tactile receptive fields restricted to a single body segment. On the other side, we put forward that the dynamic formation of a whole body gestalt is functionally tied to neuronal populations in the ventral premotor cortex

whose visuo-tactile receptive fields extend to encompass the boundaries of multiple body segments or the entire body.

Disclosures: G. Gentile: None. **M. Björnsdotter:** None. **V.I. Petkova:** None. **Z. Abdulkarim:** None. **H.H. Ehrsson:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.09/III12

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01EY012440

Title: Stimulus-locked activation of the extrastriate body area during tactile stimulation of the viewed hand

Authors: V. OCCELLI[1], R. STILLA[1], S. LACEY[1], M. LONGO[2], P. HAGGARD[3], *K. SATHIAN[4];
[1]Neurol., Emory Univ., Atlanta, GA; [2]Birkbeck Col., London, United Kingdom; [3]Inst. of Cognitive Neurosci., Univ. Col., London, United Kingdom; [4]Dept Neurol., Emory Univ. Sch. Med., ATLANTA, GA

Abstract: In the visual enhancement of touch (VET) effect, non-informative vision of the hand enhances tactile acuity relative to viewing a non-hand object (Taylor-Clarke, Kennett, & Haggard, Curr Biol 12:233-236, 2002). Although several studies have replicated the VET effect, its neural correlates remain unclear. In the present study, event-related functional magnetic resonance imaging was used to examine neural activation patterns while participants discriminated the orientation of tactile gratings applied to the right index fingerpad. Grating spatial parameters were selected for individual participants to be close to the threshold for orientation discrimination. In separate runs, participants continuously viewed either their own hand (without vision of the stimulated site) or a non-hand object. Data from separate localizer runs were used to identify the extrastriate body area (EBA), a region known to be highly responsive to the images of human bodies or body parts (Downing et al., Science 293: 2470-2473, 2001). We found bilateral EBA activity time-locked to tactile stimulation in the hand-view condition, compared to the object-view condition. In addition to this novel finding, similar activation was also observed in the intraparietal sulcus (IPS), consistent with prior evidence implicating the IPS in the VET effect (Konen & Haggard, Cerebral Cortex, in press). Our findings suggest a possible multisensory function for the EBA in processing body-related inputs.

Disclosures: V. Occelli: None. **R. Stilla:** None. **S. Lacey:** None. **M. Longo:** None. **P. Haggard:** None. **K. Sathian:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.10/III13

Topic: F.01. Human Cognition and Behavior

Support: NIH: R01 EY012440

Title: Preferences for integrative versus schematic sensory imagery across modalities

Authors: *S. A. LACEY, H. FENG, E. CAESAR, M. BHUSHAN, T. JOHN, K. SATHIAN; Emory Univ., Atlanta, GA

Abstract: Visual imagery can be divided into object and spatial subtypes. Object imagery involves pictorial images that integrate surface properties, such as color and texture, with structural information about shape. By contrast, spatial imagery involves more schematic images that tend to ignore surface properties and instead focus on structural information and spatial transformations. Using tasks in which people had to haptically discriminate object shape across changes in texture and vice versa, we showed that these imagery preferences also exist in haptically-derived representations, with haptic performance tending to reflect self-reported visual imagery preferences (Lacey, Lin & Sathian, Exp Brain Res, 213:267-273, 2011). Here, we tested the hypothesis that there are corresponding subtypes of auditory imagery. In one task, we tested whether people could discriminate the auditory structural property of melody across changes in the auditory surface property of loudness. Poor performance would indicate that structural and surface properties are integrated in the image and reflect the auditory equivalent of object imagery. In a second task, people had to discriminate loudness patterns across changes in melody. Poor performance here would indicate that surface properties were not attended and reflect the auditory equivalent of spatial imagery. Participants also completed the Object-Spatial Imagery and Verbal Questionnaire (OSIVQ, Blazhenkova & Kozhevnikov, Appl Cognit Psychol 23:638-663, 2009) and a self-report on imagery preference. We found that, as hypothesized, auditory imagery could be divided into two subtypes: ‘integrative’ imagers incorporated the loudness pattern into their representation of the melody (equivalent to visuo-haptic ‘object’ imagery) whereas ‘schematic’ imagers focused on the melody at the expense of loudness patterns (equivalent to visuo-haptic ‘spatial’ imagery). Preliminary correlation analyses suggest that imagery preferences are stable across the visual and haptic modalities - i.e. a visual object imager

tends also to be a haptic object imager - perhaps because vision and touch can both assess multiple object properties, e.g. shape and texture. However, these preferences did not necessarily extend to the auditory modality, perhaps because auditory stimuli have less in common with visual or haptic stimuli. Whether or not surface properties are integrated into an image may be therefore be an organizing principle of mental imagery across sensory modalities. In order to encompass the three modalities, we suggest that the 'object' and 'spatial' imagery labels be replaced with 'integrative' and 'schematic'.

Disclosures: S.A. Lacey: None. **H. Feng:** None. **E. Caesar:** None. **M. Bhushan:** None. **T. John:** None. **K. Sathian:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.11/III14

Topic: F.01. Human Cognition and Behavior

Support: EURYI Award to RV

BZ supported by Studienstiftung des deutschen Volkes (German National Academic Foundation)

Title: Perceptual phase entrainment to speech rhythm in the absence of spectral energy fluctuations

Authors: B. ZOEFEL, *R. VANRULLEN;
Ctr. de Recherche Cerveau et Cognition, CNRS, Toulouse, France

Abstract: Speech can be defined by its spectral content (e.g. fundamental frequency and harmonic "formants") and its variable amplitude over time. The fluctuations in amplitude and spectral content occur rhythmically (with a dominant frequency in the theta-band, 2-6 Hz). Recent evidence suggests that neural oscillations in auditory cortex entrain to the speech rhythm, resulting in an alignment of high excitability phases with informative features. However, mechanisms of phase entrainment are still unclear, because in a standard speech sequence the phonetic information (carried by momentary deviations in spectral content), is generally confounded with overall amplitude modulations: There is no phonetic information when spectral energy is absent; and there are moments of silence or low spectral energy between successive phonemes. Therefore, it is not clear whether brain oscillations truly align to fluctuations in phonetic information (a high-level process), or merely to the rhythmic changes in input sound

amplitude (a low-level process).
Here, we disentangle these alternatives by constructing speech stimuli whose spectral energy is constant over time. Original speech snippets were recorded by a male speaker reading parts of a novel. Stimuli were constructed by merging speech with noise, with the amount of noise inversely proportional to the instantaneous energy of the speech. Critically, the noise was designed to have an average spectrum equivalent to the average spectrum of the speech (i.e. fundamental frequency and harmonics). Consequently, total energy was constant over time, and spectral content was statistically comparable at all points in time - but some points merely contained noise while others contained real phonemes. Phonetic information still fluctuated rhythmically at ~2-6Hz, providing potential means for oscillatory phase entrainment. We assessed this entrainment by presenting auditory clicks at threshold level at random moments during our speech/noise snippets. Subjects indicated detection of a click by a button press. We found that detection was best at phases corresponding to original phonetic information and decreased continuously, with worst performance at opposite phases where noise was dominant. Yet by construction, both spectral content and amplitude of the speech/noise sequence were statistically indistinguishable across the different rhythmic phases. Thus, we demonstrate that the auditory threshold can be entrained by the rhythm of speech even when phonetic information is not accompanied by concomitant changes in input sound amplitude. In other words, phase entrainment to speech rhythm entails a high-level process.

Disclosures: B. Zoefel: None. **R. VanRullen:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.12/III15

Topic: F.01. Human Cognition and Behavior

Title: Handedness and Perspective during action recognition: Towards a neurophysiological model of action simulation

Authors: *R. KELLY, C. MIZELLE, L. WHEATON;
Georgia Inst. of Technol., Atlanta, GA

Abstract: During daily behavior, the ability to comprehend skilled action is an important aspect to understanding the world around us. Event-related desynchronization (ERD) in the Mu band (8-10 Hz) can be associated with increased cortical activity and can show patterns in motor tasks in the sensory motor system. An event-related synchronization (ERS) may represent inhibited

cortical activity. The purpose of this neurobehavioral study is to evaluate the interaction of handedness and perspective and how it plays a role in our ability to recognize the goals of motor acts. Right-handed subjects were first trained directly with different tools. Electroencephalography (EEG) was being recorded while the subjects were presented with randomly organized static visual images of tools from egocentric or allocentric perspectives performed by either a left or right hand. The results showed significant left lateralized sensorimotor ERD for right hand matched action simulation conditions (Right-handed/Egocentric, Left-handed/Allocentric images). For left hand matched conditions, more bilateral sensorimotor ERD was seen (Left-handed/Egocentric, Right-handed/Allocentric images). This suggests activation of motor representations of the observer's left hand when watching a left-handed person for comprehending actions, dependent of the perspective of the action seen. Consequently, the current research will help us better understand how we encode action and recall motor simulations and may ultimately contribute to understanding of some tool-use related deficits.

Disclosures: R. Kelly: None. **C. Mizelle:** None. **L. Wheaton:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.13/III16

Topic: F.01. Human Cognition and Behavior

Support: Dutch Science Foundation Talent Scheme Veni award 451-11-002

Economic and Social Research Council UK Future Research Leaders award F003639

Title: The impact of sensorimotor experience on affective evaluation of movement

Authors: *L. KIRSCH[1], K. A. DROMMELSCHMIDT[2], K. DAWSON[1], E. S. CROSS[1]; [1]Sch. of Psychology, Bangor Univ., Bangor, United Kingdom; [2]Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Prior research on aesthetics demonstrates that people are more likely to judge a stimulus as pleasing if they are familiar with. While general familiarity and liking appear to be related, it is less clear how motor familiarity, or embodiment, is related to a viewer's aesthetic appraisal of a stimulus. Previously, we demonstrated that people derive greater pleasure from watching movements they are not able to embody compared to those they felt they could. To more closely investigate this finding, we subsequently conducted two dance training experiment

to clarify the relationship between sensorimotor experience and affective evaluation of a movement. The first experiment used a between-subjects design wherein 62 participants were assigned to one of three groups and took part in four days of training. One group received physical training on simple dance sequences with the Xbox KinectTM system, another visual and auditory training, and a third group received auditory experience only. Participants' aesthetic preferences for different dance stimuli were measured with a rating task before and after the training sessions. Results demonstrate that participants from the physical training condition not only improved their performance of the dance sequences, but they also reported higher enjoyment and interest in the sequences after training. These results suggest that learning to embody particular movements can indeed lead to greater enjoyment while watching those movements in the future. The second experiment is an fMRI version of Experiment 1 that directly compared how learning to embody an action impacts the neural response when watching and aesthetically evaluating that action. Using a within-subjects design, 24 participants trained for four consecutive days on dance sequences set to music videos. Each day they physically rehearsed one set of dance sequences, passively watched a second set of sequences, and listened to the music of a third set. Functional MRI was obtained prior to and immediately following the four days of training, as well as affective and physical ability ratings. This approach enables us to examine the brain basis of how action embodiment is related to the pleasure a viewer derives from watching an action. Moreover this study enables a precise comparison of self-report methods of embodiment with non-biased, empirical measures (e.g., scores given by the participant vs. the Kinect system). Together, these two studies are the first to use intensive training procedures coupled with fMRI to investigate affective judgment in relation to embodiment, and will inform both affective and motor research areas.

Disclosures: L. Kirsch: None. **K.A. Drommelschmidt:** None. **K. Dawson:** None. **E.S. Cross:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.14/III17

Topic: F.01. Human Cognition and Behavior

Support: NWO Spinoza

Title: The behavioral and neural effects of language on motion perception

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Abstract: Perception does not function as an isolated cognitive system but is actually tightly linked with other cognitive functions. Several studies have described an influence of language on motion perception, but it remains debated at which level of processing this modulation takes place. Some studies argue for an interaction in perceptual areas, by showing that language can impact on perceptual sensitivity as well as modulate neural activity in sensory cortex during a perceptual task. A second possibility would be that the interaction is mediated by 'language areas' that integrate the semantic meaning of the linguistic as well as the visual information. Here, we investigated this issue in a combined behavioral and fMRI study. Moreover, we assessed whether language-perception interactions were specific to the language-dominant left hemisphere, by presenting visual material to the right and left visual field.
Subjects (right-handed; behavior: N=22; fMRI: N=25) performed a visual motion detection task. On each trial, the visual motion stimulus was presented in either the left or in the right visual field (RVF). Crucially, subjects were shortly presented with a motion word (e.g., rise) before each trial. The motion word could be congruent, incongruent or neutral with regard to the visual motion stimulus that was presented subsequently.
When motion words were congruent with the direction of the visual motion stimulus, subjects were faster, more accurate, and had a more liberal decision threshold compared to the incongruent condition. Interestingly, the speed benefit was present only for visual stimuli that were presented in the RVF. We observed a neural counterpart to these behavioral facilitatory effects in the left middle temporal gyrus (lMTG), an area involved in semantic matching and contextual integration, but not in the visual areas that were sensitive to the motion stimulus.
In conclusion, we show that language affects behavioral performance on motion perception, with stronger effects for motion stimuli that are processed in the language-dominant left hemisphere. These interactions were neurally mediated by 'language areas' rather than perceptual areas, suggesting that these form part of the network involved in perceptual decisions about visual motion stimuli.

Disclosures: J.C. Francken: A. Employment/Salary (full or part-time):; Donders Institute for Brain, Cognition and Behavior, Radboud University Nijmegen, Nijmegen, The Netherlands. **P. Kok:** A. Employment/Salary (full or part-time):; Donders Institute For Brain, Cognition and Behavior, Radboud University Nijmegen. **P. Hagoort:** A. Employment/Salary (full or part-time):; Donders Institute For Brain, Cognition and Behavior, Radboud University Nijmegen. **F.P. De Lange:** A. Employment/Salary (full or part-time):; Donders Institute for Brain, Cognition and Behavior, Radboud University Nijmegen, Nijmegen, The Netherlands.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.15/III18

Topic: F.01. Human Cognition and Behavior

Title: Decoding actions across objects

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Abstract: Action perception likely involves the neural activation of several distinct representational levels, e.g., the kinematics vs. the goals of an action. Actions, however, can also be described in terms of their conceptual content. Action concepts are generalizations of concrete actions. For example, the action concept "cutting" is a common, perceptually independent representation of the two actions "cutting an apple" and "cutting a potato". To date, however, the neural substrates of action concepts are still unclear. Here we studied how conceptual representations of observed actions are organized in the brain using multivariate pattern analysis (MVPA) of fMRI data. Fourteen participants observed two different actions (cutting, peeling) that involved two different objects (apples, potatoes). This design allowed identifying object-independent representations of the action concepts "cutting" and "peeling". This was done by training a support vector machine (SVM) classifier to discriminate the two actions in one object (apple) and testing the classifier with the two actions in the other object (potato), and vice versa. To avoid confounds on a perceptual level, we used five exemplars of each action performed by two different actors, thus enhancing the perceptual variance of the stimuli. Participants were instructed to perform a one-back task on either the actions or the objects. We found significant above-chance classification accuracies for action concepts in posterior middle temporal gyrus (pMTG). This result substantiates previous findings on the role of the pMTG in representing perceptually invariant action information and suggests that observed actions are generalized to conceptual representations of actions in occipitotemporal cortex.

Disclosures: M.F. Wurm: None. **G. Ariani:** None. **M.W. Greenlee:** None. **A. Lingnau:** None. **S. Petris:** None.

Poster

571. Perception: Auditory, Tactile, and Multisensory

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.16/III19

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH092345

NIH Grant T32 MH020002

Title: Temporal dynamics of human perceptual decision making: Fine discriminations are guided by the activity of the most informative sensory neurons

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Abstract: Visual perception can be characterized as a process of inference: given a set of noisy neural responses, what is the state of the external world? For coarse discriminations (e.g., when discriminating between two orthogonal orientations), a good strategy is to query the responses of neurons that are tuned to the relevant alternatives, as those neurons undergo the largest change in firing rate. However, the same strategy is suboptimal for fine discriminations (e.g., when discriminating between two very similar orientations), as neurons that are selective for feature values further away from the relevant alternatives are more sensitive to small changes in the stimulus. Here, we recorded EEG while human subjects discriminated small clockwise or anticlockwise rotations in stimulus orientation in order to determine if fine perceptual discriminations were influenced by these informative "off-channel" neurons. EEG responses were wavelet transformed, and we used a multivariate encoding model to estimate a trial-by-trial orientation selective response profile with a temporal resolution of 50-100 msec. (see Garcia et al., Cur Biol 23:515-522). Critically, in the responses of orientation channels tuned slightly away from the target were larger than those tuned to the target on correct relative to incorrect trials, particularly in the temporal interval immediately preceding the behavioral response. These findings support the hypothesis that perceptual decisions are based on the activation level of the most informative sensory neurons, and suggest that sensory neurons do more than 'vote' for their preferred feature. Instead, the activity of sensory neurons can be decoded in a more optimal manner that exploits their sensitivity to detect small changes away from their preferred feature.

Disclosures: E.F. Ester: None. **J.T. Serences:** None.

Poster

571. Perception: Auditory, Tactile, and Multisensory

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.17/III20

Topic: F.01. Human Cognition and Behavior

Support: MH081018

EY017491

EY021553

Title: Quick adaptive Bayesian assessment of sensory memory decay

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Abstract: Sensory memory is the literal, modality-specific neural representation of sensory stimuli in the human brain (Sperling, 1960). It provides the initial copy ("buffer") of external stimulation to human sense organs that can be processed by subsequent stages of perception and cognition. Recent studies suggest that sensory memory decays much faster for observers with mild cognitive impairment and may serve as an early sign of the Alzheimer's disease (Lu et al., 2005). In the visual modality, iconic memory is best assessed with the partial report procedure. In this procedure, an array of letters appears briefly on the screen. A post-stimulus cue directs the observer to report the identity of the cued letter. Typically 6-8 cue delays or 600-800 trials are tested to measure the sensory memory decay function. The procedure takes about one hour. The long testing time has prevented wide use of the test in clinical settings and special populations. Here we develop a quick partial report or qPR procedure based on a Bayesian adaptive framework to directly estimate the parameters of the sensory memory decay function with much reduced testing time. In the qPR, parameters of the exponential decay function are characterized by probability distributions. Starting with a prior distribution of the parameters, the method selects the stimulus to maximize the expected information gain in the next test trial. It then updates the probability distribution of the parameters based on the observer's response by Bayesian inference (Kontsevich & Tyler, 1999; Lesmes et al., 2006). The procedure is iterated until either the total number of trials reaches a set value or the precision of the parameter estimates reach a certain criterion. Simulation studies suggest that only 100 trials are necessary to reach 1dB accuracy and 2dB precision. The method was validated in a psychophysical experiment. Estimates of the sensory memory decay function obtained with 100 qPR trials showed good precision (SD=0.6 dB) and excellent agreement with those obtained with 1600 trials using the conventional procedure (mean RMSE =1dB). qPR relieves the data collection burden in characterizing sensory memory and makes it possible to assess sensory memory in clinical settings and special populations.

Disclosures: J. Baek: None. **L. Lesmes:** None. **Z. Lu:** None.

Poster

571. **Perception: Auditory, Tactile, and Multisensory**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 571.18/III21

Topic: F.01. Human Cognition and Behavior

Title: Gender differences in influence of sound environments on performance of the memorizing numerical string task and cerebral blood flow changes

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Abstract: [Purpose]
In the former studies, it was demonstrated that sound environments affect the results of intellectual work and cerebral blood flow changes using functional near-infrared spectroscopy (fNIRS). Furthermore, psychology studies have reported that gender-specificity exists in the influence of pleasant and unpleasant sounds on the work environment. In this study, we investigated gender difference in the influence of sound environments by scoring a memorizing numerical string task and observing cerebral blood flow changes.
[Methods]
This study examined 24 subjects, among which 12 were males and 12 females. In this study, we selected three sound environments, namely, silence; "the sonata for two pianos in D major, K.448" by Mozart; and white noise. Subjects were exposed to sounds while working. The effect on intellectual work was analyzed by measuring the number of correct answers to 30 questions. Subjects had to memorize eight numbers in 3 sec and input them in the correct order in <7 sec. We examined the influence of different sound environments on the task by measuring cerebral blood flow changes using fNIRS.
[Results]
The results revealed that males showed the best performance when exposed to silence, whereas females showed the best performance in the presence of white noise. The influence of sound environments on the score was significant at 5% level in both males and females as calculated using the two-way factorial ANOVA without replications. Simultaneously, the results of the t-test at 5% level showed significant differences between males and females only in the presence of white noise. In addition, the same significant difference was observed in the average cerebral blood flow change. Cerebral blood flow change in males decreased and that in females increased. Moreover, it was observed that cerebral blood flow change decreases under pleasant sound conditions and increases under unpleasant conditions. Also in this experiment, in the white noise, cerebral blood flow change in males was tended to decrease and that in females tended to increase. Therefore, we suggest that males may feel that the white noise is unpleasant as opposed

to females. In conclusion, females showed the best performance in the presence of white noise, under which their cerebral blood flow change increased. Based on these results, we found that there is a gender-specific difference in the performances of intellectual work and cerebral blood flow changes in the presence of white noise.

Disclosures: A. Masazumi: None. **U. Yamamoto:** None. **T. Hiroyasu:** None.

572**Poster**

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.01/III22

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1157432

Vanderbilt Discovery Grant

Title: Incorporating neural signals into computational models of memory search

Authors: *S. M. POLYN[1], N. W. MORTON[2], J. E. KRAGEL[3], J. D. MCCLUEY[2];
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Abstract: A major goal of cognitive neuroscience is to understand how neurally recorded signals map onto psychological processes within a formal modeling framework. These frameworks are interactive systems that potentially allow us to simultaneously assess the diverse functions of distinct brain regions. In the domain of memory search, activity in one region may relate to association formation, activity in another region may reflect integrative processes, and these processes may interact during both study and recall. Exploring the dynamics of these signals requires models that can incorporate both neural data and behavioral data. Because neural data inherently varies on an item-by-item basis, these models must be specified at the single-item level; summary statistics such as serial-position curves, lag-based conditional response probability analysis, and various organizational measures remove the item-by-item variability one wants to explain. In the domain of memory search, trial-level data are in the form of recall sequences: An ordered series of responses made by a participant during the recall period. I will describe a technique that allows one to calculate the likelihood that a given recall sequence is observed, from among all possible sequences that could be produced by a given computational model. The technique does not require that the computational model is stationary; the model parameters can change both during the study period and during memory search. Analysis of synthetic neural data will be presented to demonstrate how this approach allows one to compare

various hypotheses linking neural signal to behavioral data, even in the presence of multiple interacting mechanisms at study, and a non-stationary recall process. I will also demonstrate the utility of this approach in the behavioral domain, in particular, when dealing with results that reflect multiple interacting mechanisms (such as those underlying temporal, semantic, and source organization in free recall). Certain limitations of the approach will also be discussed, with an eye towards the eventual integration of this approach with modern Bayesian techniques.

Disclosures: S.M. Polyn: None. **N.W. Morton:** None. **J.E. Kragel:** None. **J.D. McCluey:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.02/III23

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1157432

Title: Representation of item and context specific information during memory retrieval in the human brain

Authors: *J. E. KRAGEL, S. M. POLYN;
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Abstract: Recent neurocognitive theories of memory retrieval attribute the subjective phenomenon of recollection to the reactivation of patterns of neural activity present during the formation of an encoded memory. This distributed cortical activity is thought to reflect multiple facets of the encoded event, including item and context specific information. While the capacity of ventral temporal and primary sensory cortices to represent stimulus-specific information is well established, the neural mechanisms that support the representation of contextual information are less well characterized. Retrieved-context theories propose a slowly changing, autocorrelated temporal context signal that contributes to the episodic nature of an encoded event. While previous studies of electrophysiological recordings in humans and rodents have identified temporal-context like signals within the medial temporal lobe, autocorrelated noise in functional magnetic resonance imaging (fMRI) signal presents a challenge to identifying neural regions that may contain such a contextual signal. Using a simulation based approach, we test the ability to identify meaningful autocorrelated signal in the fMRI timeseries. Using retrieved context models as a framework, we demonstrate the capacity to detect the presence of a stimulus-driven contextual signal in the presence of autocorrelated noise. Applying these techniques to fMRI

studies of episodic memory retrieval, we test the capacity of specific cortical to regions to represent contextual and item specific information.

Disclosures: J.E. Kragel: None. **S.M. Polyn:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.03/III24

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1157432

Vanderbilt Discovery Grant

Title: Inter-item distraction dissociates temporal and semantic organization in free recall

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Abstract: Retrieved-context models propose that information related to studied items is integrated into a representation of temporal context, which later serves as a cue to probe memory. Consistent with these models, recent scalp EEG results suggest that item-category-specific information is integrated over time. Furthermore, the rate of this integration predicts the degree to which recalls will subsequently be clustered by stimulus category. Previous findings suggest that category clustering is attenuated when a distracting task is performed between items. We hypothesized that distracting activity disrupts temporal context, causing category-specific information to accumulate less over time; as a result, recall is less influenced by category cues. To test this account, we examined recall behavior and oscillatory scalp EEG data in free recall trials with or without distracting activity during study. We found that distraction greatly attenuated category clustering. In contrast, temporal organization, the tendency to successively recall items presented near to each other, was unaffected by distraction. Using multivariate pattern analysis, we examine whether neural signatures of integrative activity differ between the two conditions, and whether differences in integrative activity during encoding predict the degree to which category clustering is attenuated.

Disclosures: N.W. Morton: None. **S.M. Polyn:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.04/III25

Topic: F.01. Human Cognition and Behavior

Support: NWO Grant 433-09-239

Title: Consolidation of newly learned words: Does the presence of pictures at encoding make a difference?

Authors: *A. TAKASHIMA[1,2], I. BAKKER[1,2], J. G. VAN HELL[3,2], G. JANZEN[1,2], J. M. MCQUEEN[1,2,4];
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Abstract: From lesion and imaging studies, we know that memory is not a unitary system. Declarative memory is considered to entail episodic memory (memory for episodes that are confined to specific spatial and temporal contexts) and semantic memory (memory for generic knowledge or concepts). Although these two types of memories are not independent and highly interactive, they seem to involve different brain structures at retrieval, with the hippocampus often regarded to be important for retrieving arbitrary associative information encoded in a specific episodic context, whereas widely distributed neocortical areas, especially higher order associative areas, seem to be important in retrieving semantic or conceptual information.
In this study, we were interested in the neural correlates of newly learned words when retrieved at different time points. We asked if there is more involvement of the episodic memory network when retrieval occurs directly after learning, and if there is a shift towards more involvement of the semantic network as the word becomes more de-contextualized with time. Furthermore, we were interested to see the effect of having extra information at encoding, namely, visual information associated with the phonological form of the novel word. Previously we have reported that picture-associated novel words retrieved immediately after encoding involve hippocampal structures more than phonological form-only words. When retrieval took place 24 hours later, the involvement of the hippocampal system was still present for the picture-associated words, but involvement of distributed neocortical areas also increased with time.
In the present study, we extended the delay from 24 hours to 1 week. Participants learned phonological novel word forms with/without corresponding pictures and their memory for the words was tested in an fMRI scanner. Retrieval success was greater for picture-associated words compared to phonological form-only words on both recent and remote test. When memory for

the associated picture of the word was tested, exactly the same pictures were recognized faster than similar pictures at both time points, but at remote test we also observed an interaction. Specifically, although recognition of the same pictures slowed down as a function of delay, this was not the case for similar pictures, indicating decay of the episodic memory trace and some de-contextualization of the word memory. On the neural level, the memory benefit of having pictures at encoding was observed as more involvement of the hippocampus during recent retrieval, and increased activity in the fusiform gyrus and the left angular gyrus during delayed retrieval.

Disclosures: A. Takashima: None. **I. Bakker:** None. **J.G. van Hell:** None. **G. Janzen:** None. **J.M. McQueen:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.05/III26

Topic: F.01. Human Cognition and Behavior

Support: ERC Grant 30000075

Title: Schema effects on spatial associative memory in humans

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Abstract: Activated neural representations of prior knowledge, or "schemas", are found to facilitate assimilation of congruent information in both humans and rats. This schema-related facilitation is thought to be mediated by interactions between the medial prefrontal cortex (mPFC) and hippocampus. In rats, the existence of a spatial schema enabled rapid learning of new flavor-location associations. Moreover, retrieval of these associations rapidly became hippocampally independent and was mediated by the mPFC (Tse et al. 2007, Science 316, 76-82; Tse et al. 2011, Science 333, 891-895). In this fMRI study, we investigate whether the existence of such a spatial schema facilitates learning and retrieval of new object-location associations in humans and whether retrieval of these associations is mediated by the mPFC.
To this aim, we developed a human analogue of the event arena used by Tse and colleagues. The task consists of two conditions in which subjects are instructed to memorize object-location associations within two different 10 x 10 grids. In the schema condition, the associations remain

consistent over sessions, whereas in the no-schema condition the objects and possible locations are fixed, but the associations are flexible across sessions. Subjects are initially trained on 50 associations per condition on three consecutive days. On day 4, 50 new object-location associations are introduced per condition in addition to the old ones. 24 hours later, recall for both old and new associations is tested while brain activity is measured using fMRI.
Up to now, data acquisition is finalized for 17 out of 24 subjects. Behavioral results show an increase in learning over sessions for schema associations and within a session for no-schema associations. On average, schema associations were well learned at the end of day 3 (94.7% correct). As expected, during the recall session on day 5, subjects showed better memory for new schema associations (63.1%) compared to new no-schema associations (53.3%) (t (16)= 4.5, p<.0005), indicating that a spatial schema was developed. Reaction times for new associations did not differ significantly between the two conditions (p=.115). Next, we will analyze brain activity during recall using univariate regression analyses to investigate the neural mechanisms underlying schema-related retrieval.
Our findings are in line with the studies of Tse and colleagues and indicate that the presence of a spatial schema facilitates learning and memory of new, related associations in which objects are linked to specific locations.

Disclosures: M. Van Buuren: None. **G. Fernández:** None. **M. Kroes:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.06/III27

Topic: F.01. Human Cognition and Behavior

Support: NWO 918.66.613

ERC-2010-AdG 268800

Title: Evidence for reconsolidation of emotional episodic memories in humans

Authors: *M. C. KROES[1], I. TENDOLKAR[2], G. A. VAN WINGEN[3], J. A. VAN WAARDE[4], B. A. STRANGE[5], G. FERNÁNDEZ[6];
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Madrid, Spain; [6]Donders Inst. for Brain, Cognition, and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: The reconsolidation hypothesis postulates that upon reactivation, memory traces return to a labile state and are once again susceptible to disruption by treatments similar to those that impair initial memory consolidation. Despite accumulating evidence for a reconsolidation process in animals, support in humans, especially for episodic memory is limited. This discrepancy is largely due to the fact that animal studies on reconsolidation have generally employed invasive techniques to disrupt reconsolidation, which cannot be easily translated to humans. Studies on human reconsolidation have therefore used either systemic application of slow-acting pharmacological compounds, or psychological manipulations such as interference learning. As a result, human studies often have had difficulty meeting critical criteria to show that reconsolidation is a time-dependent process, or are susceptible to alternative interpretations such as interference at the time of retrieval. Here we circumvent these issues by showing that a single application of electroconvulsive therapy (ECT) including short-term narcosis following memory reactivation in patients with unipolar depression disrupts reactivated, but not non-reactivated, memories for an emotional episode in a within-subjects design. This effect is not visible immediately, but only after a 24h delay. Thus, it is time-dependent satisfying a critical reconsolidation criterion. In conclusion, we provide causal evidence for reconsolidation in humans provided by direct interference with neural activity following memory reactivation. This finding provides a proof-of-principle and opens potential therapeutic avenues targeting reconsolidation to alter memories that contribute to the persistence of psychiatric disorders.

Disclosures: M.C. Kroes: None. **I. Tendolkar:** None. **G.A. van Wingen:** None. **J.A. van Waarde:** None. **B.A. Strange:** None. **G. Fernández:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.07/III28

Topic: F.01. Human Cognition and Behavior

Support: ERC Grant 30000075

Title: Probing rule-based schematic memory representations

Authors: *I. WAGNER[1,2], M. VAN BUUREN[1,2], M. VAN DER LINDEN[1,2], M. KROES[3], G. FERNÁNDEZ[1,2];
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Abstract: Neural representations of existing knowledge, skills and attitudes, so-called "schemas", can alter the manner in which information is processed, including memory encoding, consolidation and retrieval. This processing is thought to be mediated by interactions between the medial temporal lobe (MTL) and medial prefrontal cortex (mPFC). However, it is unclear whether these areas actually hold schematic representations. Here, we studied schema formation based on two distinct sets of rules and investigated whether neural signatures of these two schemas are dissociable within MTL and mPFC.

Using functional MRI, brain activity was measured while participants learned to apply two sets of rules (spatial vs. non-spatial) in a deterministic weather prediction task (Kumaran, 2009), in which simple shapes were associated with a weather outcome. The experiment consisted of two sessions on consecutive days. On Day 1, participants performed both encoding and recall trials. During encoding trials, feedback about the weather outcome was provided, whereas during interspersed recall trials no feedback was given and participants did not have the chance to improve. Across the first session, these recall trials yield a graded measure of actual schema proficiency. On Day 2, participants solely completed recall trials. Additionally, they were required to transfer their schema knowledge to new stimuli to test for their ability to apply the schemas.

On average, participants reached almost perfect performance (>90% correct) within the first four runs of Day 1 (n=23). Performance did not differ significantly between the two schema conditions (Encoding: $t_{22}=-0.989$, ns.; Recall: $t_{22}=-2.003$, ns.). To investigate general consolidation processes with a univariate analysis, we compared recall trials between the two sessions (Day 2>Day 1). Ventromedial PFC showed increased activation on Day 2 as compared to Day 1 ($p<.05$, SVC), which cannot be explained by differences in task difficulty (indicated by % correct responses) between sessions ($t_{22}=-0.642$, ns.). This finding is in line with previous findings and supports the notion that schema consolidation is mediated by the mPFC.

To closer investigate differences in neural representations between the two schemas, we will apply multivariate pattern analysis (MVPA). Here, we will test consolidated schema-patterns and their generalisability to neural representations during a transfer session. Furthermore, we will be tracing the distinct neural schema-patterns throughout their formation and assume to find differential classifier performance for schema discrimination within MTL and mPFC over time.

Disclosures: I. Wagner: None. **M. van Buuren:** None. **M. van der Linden:** None. **M. Kroes:** None. **G. Fernández:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.08/III29

Topic: F.01. Human Cognition and Behavior

Support: Department of Defense in the Center for Neuroscience and Regenerative Medicine

Intramural Research Program of the NINDS, NIH

Title: The critical role of prefrontal cortex in strengthening of episodic memories through reconsolidation

Authors: *M. SANDRINI, N. CENSOR, J. MISHOE, L. COHEN*;
NINDS-NIH, Bethesda, MD

Abstract: Consolidation theory characterizes consolidation as a one-time event, after which memories become resistant to interference. Research into memory reconsolidation has challenged this view. Reactivation of consolidated memories opens the reconsolidation window, a time-limited period during which memories can be modified. However, it is unknown whether neuromodulation of neocortex during the reconsolidation window might strengthen human memories. Episodic memory refers to long-term declarative memory for specific events in space and time. To neuromodulate this memory during the reconsolidation window, we applied repetitive Transcranial Magnetic Stimulation (rTMS) to right prefrontal cortex (PFC), a region involved in episodic memories.

Fifty human subjects participated in this study. Subjects learned a list of 20 words on Day 1. On Day 2, memory was reactivated by a contextual reminder cue and 10 minutes later 1 Hz rTMS was applied for 15 minutes to right PFC (PFC-R), a control site (Vertex-R), or right PFC without a reminder cue (PFC-NR). Memory recall was tested on Day 3 (24h post-reactivation, Exp. 1) or on Day 2 (1h post-reactivation, Exp. 2)

We report that non-invasive stimulation of right prefrontal cortex (PFC-R) during reconsolidation strengthened episodic memories, an effect documented by improved recall 24h post-reactivation compared to control groups (i.e. PFC-NR and Vertex-R). In contrast, there was no effect of stimulation 1h post-reactivation, showing that memory strengthening is time-dependent, consistent with the reconsolidation theory. Thus, prefrontal cortex plays a critical role in strengthening of episodic memories through reconsolidation. Reconsolidation may serve as a window of opportunity to strengthen human episodic memories with non-invasive neuromodulation, an issue of relevance for clinical applications.

Disclosures: M. Sandrini: None. **N. Censor:** None. **J. Mishoe:** None. **L. Cohen*:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.09/III30

Topic: F.01. Human Cognition and Behavior

Support: PSC-CUNY 65054-0043

Title: Knowing you know: A transcranial direct current stimulation (tDCS) study of the feeling-of-knowing in episodic memory

Authors: *E. F. CHUA[1,2], S. MEYLER[1];
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Abstract: When people fail to recall information, they sometimes have a feeling-of-knowing (FOK) that the currently non-recallable item is known and/or will be later remembered. Several previous neuroimaging studies have implicated the prefrontal cortex in the subjective level of feeling-of-knowing expressed. Because fMRI is correlational, the aim of this study was to test for a causal role of the prefrontal activity in the feeling-of-knowing. Transcranial direct current stimulation (tDCS), a non-invasive form of brain stimulation, was used to stimulate the PFC during a memory task. Participants studied 180 faces and names. Following study, they either received active prefrontal stimulation (1.5 mA for 10 minutes) or sham stimulation. At test, participants were presented with a face and asked to recall the name. Following recall, they made a FOK judgment and indicated on a scale of 1-6 whether they thought they would recognize the correct response. They then complete a 3 alternative forced choice recognition test for the name associated with the face. One week later, participants returned to the lab and completed the same task using a different set of faces and names and received either active or sham stimulation, whichever one had not been received in visit 1. Preliminary analyses (n=21) showed that participants gave significantly lower FOK judgments for face-name pairs that were later correctly recognized during active compared to sham stimulation ($p<0.05$). These results demonstrate that directly manipulating prefrontal activity has a causal effect on the level of FOK expressed.

Disclosures: E.F. Chua: None. **S. Meyler:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.10/III31

Topic: F.01. Human Cognition and Behavior

Title: Visual evoked potentials in humans are enhanced by long-term visuo-gustatory conditioning

Authors: I. VIEMOSE, C. LILJENDAHL, J. L. LAUGESEN, P. MOELLER, W. L. P. BREDIE, *G. R. CHRISTOFFERSEN;
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Abstract: Long lasting memories of associations between the visual features and tastes of objects are essential for identification of food items. However, hardly any information is available on the long-term effects of image-taste conditioning on image-induced brain potentials.
Aim: The project aimed at comparing visual evoked potentials (VEPs) induced by nonsense images presented either for the first time or after having been used as conditioned stimuli paired with gustatory (unconditioned) stimuli.
Methods: A 118 electrode EEG-array was used to record VEPs before and one day after a training session containing 3 paired stimulations with one image and an appetitive juice and 3 pairs of another image and a juice with an aversive taste. The images were unfamiliar and nonsensical 2D figures. Before and after training, images were presented for 1 sec at 3 sec intervals, 60 times each in semi-random sequence. Before and during training, images were presented in the right side visual field relative to a fixation cross (activating left posterior visual cortices). After training, the original figure plus a duplicate - placed symmetrically in the left visual field - were presented. This allowed two types of comparisons between conditioned and not conditioned states: 1) comparisons of left hemisphere VEPs before and after training and 2) comparisons after training of VEPs in the left (conditioned) and the right (non-conditioned) hemispheres. Data were analyzed in the electrodes: OI1, O1, POO9, POO3, PO9, PO7, PO5, PO3, PP09 and the corresponding right side electrodes. Results are stated as mean ± sem from 9 subjects.
Results: Conditioning altered late VEP components beginning at N2 peak and covering P3. These components were dominated by waves in the delta and theta frequency bands. After training, average theta power in the left (conditioned) side was 1.30±0.42 μV2 compared to only 0.79±0.16 μV2 in the right (not conditioned) side ($p<0.0001$). Before training, left side theta power was 0.63±0.21 μV2, significantly lower than after training ($p<0.0001$). The corresponding values for delta power were: 2.71±1.04 μV2 (left, after training), 1.31±0.33 μV2 (right, after training; $p<0.0001$) and 2.02±0.97 μV2 (left, before training; $p<0.01$). Effects of appetitive and aversive taste conditioning did not differ significantly.
Conclusions: 1) N2-P3 components of posterior VEPs showed enhanced power 24 hr after image-taste conditioning. 2) Power in the conditioned visual hemisphere was higher than in the contralateral side which had not received conditioned visual stimuli during training. The results demonstrate potentiation of the VEP induced by taste-conditioning.

Disclosures: I. Viemose: None. **G.R. Christoffersen:** None. **C. Liljendahl:** None. **P. Moeller:** None. **J.L. Laugesen:** None. **W.L.P. Bredie:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.11/III32

Topic: F.01. Human Cognition and Behavior

Title: Context memory and remembering recruit distinct neural substrates

Authors: *S. D. SLOTNICK[1], P. P. THAKRAL[2];
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Abstract: Context memory (i.e., memory for contextual information) and remembering (i.e., memory with specific detail) are assumed to reflect the same process. However, the neural substrates of these objective and subjective memory types, respectively, have been confounded in previous studies as context memory is highly correlated with remembering. The aim of the present fMRI study was to dissociate the neural substrates associated with context memory and remembering to determine whether they are mediated by the same process or by different processes. At encoding, participants were presented with colored and gray abstract shapes and were instructed to encode each shape and its context (i.e., whether it was colored or gray). At retrieval, old and new shapes were presented in gray and participants classified each shape as "old and previously colored", "old and previously gray", or "new", followed by a "remember" or "know" response. A "remember" response corresponded to memory for specific detail, whereas a "know" response corresponded to non-detailed familiarity. Item memory and context memory corresponded to accurate shape recognition and accurate memory for color or gray, respectively, while item memory alone corresponded to accurate shape recognition and inaccurate memory for color or gray. To dissociate remembering from context memory, the contrast of remember-item memory > know-context memory was conducted. To dissociate context memory from remembering, the contrast of know-context memory > remember-item memory was conducted. Neural regions specifically associated with remembering were observed in the occipital cortex and the dorsolateral prefrontal cortex. These results support previous findings that indicate the prefrontal cortex is associated with subjective aspects of retrieval. A single neural region was specifically associated with context memory in the right posterior middle temporal gyrus, which may reflect visual categorical processing. In addition, the conjunction of remember > know and context memory > item memory produced activity in the shape processing region of the occipital

cortex. The present results indicate that context memory and remembering are largely mediated by different neural processes.

Disclosures: S.D. Slotnick: None. **P.P. Thakral:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.12/III33

Topic: F.01. Human Cognition and Behavior

Title: Sleep-dependent consolidation preferentially benefits pattern separation over pattern completion

Authors: *J. R. JAMES, C. B. KIRWAN;
Psychology, Brigham Young Univ., Provo, UT

Abstract: Computational models posit that memory representations for similar inputs are made more dissimilar through a process of orthogonalization known as pattern separation. Furthermore, previously-stored memory representations may be retrieved when given a partial or degraded cue through a process known as pattern completion. These models also predict that, during sleep, the location of memory representations is gradually shifted from the hippocampus to the cortex. Cortically-mediated memory representations are more generalized, stable, and useful, but also less specific than the memory representations stored in the hippocampus. Thus, sleep-dependent consolidation should preferentially benefit pattern completion performance. Specifically, participants should be more likely to pattern complete following a sleeping delay than a waking delay. In the current study, participants studied several hundred pictures of common objects. After a twelve-hour delay, during which participants either slept or stayed awake, participants were asked to indicate whether lure images were exactly the same or merely similar to those they studied. Participants' ability to correctly reject lures was a quadratic function of target-lure similarity. A mixed ANOVA revealed an interaction of similarity and delay type, indicating that these functions significantly differed by delay type. Visual inspection of the trend lines suggests that participants are better at discriminating after sleeping than after a waking delay. These data contradict the predictions of current computational models and suggest that sleep may preferentially benefit pattern separation and not pattern completion.

Disclosures: J.R. James: None. **C.B. Kirwan:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.13/III34

Topic: F.01. Human Cognition and Behavior

Support: MEXT/JSPS KAKENHI Grant Numbers 19002010, 24220008 to Y.M.

JST, CREST to Y.M.

JSPS Research Fellowships for Young Scientists to K.M. (234682)

SPS Research Fellowships for Young Scientists to T.M. (201204982).

Title: Neural correlates of primacy and recency effects in macaque memory retrieval network

Authors: *K. MIYAMOTO[1], T. OSADA[1], Y. ADACHI[1], T. MATSUI[1,2], H. M. KIMURA[1], T. WATANABE[1], R. SETSUIE[1], Y. MIYASHITA[1,2];
[1]Dept Physiol, [2]Dept Phys, Univ. Tokyo, Tokyo, Japan

Abstract: Human functional magnetic resonance imaging (fMRI) studies have revealed involvement of a brain-wide network including the posterior parietal cortex (PPC) during memory retrieval. Investigations in nonhuman primates would help elucidate the network dynamics as well-controlled experimental techniques are available. However, a homologous memory retrieval network in the macaque brain has not been established. To identify macaque retrieval-related regions, we conducted fMRI experiments of two macaque monkeys performing a serial probe recognition task in a 4.7T MRI scanner. In this task, monkeys viewed a list of serially presented picture items and, after a delay, judged whether the test item was seen or unseen in the list. Behaviorally, a typical U-shaped serial position curve for memory accuracy accompanied by primacy and recency effects was observed in both monkeys (retrieval of initial or last item *vs.* middle item; chi-square test; $P<0.05$, Ryan's correction). We first compared cortical activity during correct recognition of previously seen items and correct rejection of unseen items, and identified 47 areas including two major PPC activation sites: the posterior inferior parietal lobule (PG/PGOp) and the intraparietal sulcus (PEa/DIP) ($P<0.01$ FDR corrected). Next, we found contrasting profiles between these two PPC regions depending on the position of retrieved items serially presented in the study list: retrieval-related activity in the PG/PGOp and hippocampus correlated with the behavioral primacy effect, while that in the PEa/DIP correlated with the behavioral recency effect ($P<0.05$, multiple regression analysis). In addition, the 47 retrieval-related regions were segregated into six subnetworks by community detection analysis using modularity optimization of the functional connectivity of spontaneous BOLD activity taken

from the two monkeys. The two PPC sites were embedded in distinct subnetworks: PG/PGOp overlapped with the monkey default-mode network, while PEa/DIP overlapped with the monkey frontoparietal network. Gross activity of the two networks during retrieval was typified by the activities of the two PPC sites with differential serial probe recognition profiles. Double dissociations of psychophysiological interactions between the two PPC sites and the hippocampus or visual cortex conformed with this network-level segregation. These results converged to reveal functional differentiation of the macaque memory retrieval network related to the primacy or recency effect, respectively. Furthermore, interspecies comparisons provided evidence of common neural substrates of memory retrieval network in monkey and human.

Disclosures: K. Miyamoto: None. **T. Osada:** None. **Y. Adachi:** None. **T. Matsui:** None. **H.M. Kimura:** None. **T. Watanabe:** None. **R. Setsuie:** None. **Y. Miyashita:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.14/III35

Topic: F.01. Human Cognition and Behavior

Support: UNAM PAPIIT Project IN304112

Title: Applying the logic of ARP´s hypermnesia hypothesis to reduce the intensity of negative emotions

Authors: *V. M. SOLIS, SR;
Psychology Dept. Natl. Univ. of Mexico, Mexico City, Mexico

Abstract: Hypermnesia occurs when memory grows across repeated trials. One tenet of the ARP´s (*alternative retrieval pathways*) hypothesis (Solís-Macías, 1998, 2008a) is enhanced hypermnesia as more pathways are activated. Pathways can be, for instance, distinct sensory modalities (visual, auditory, tactile) or conceptual categories (verb, noun, natural, human-made), among others. We applied ARP´s logic to reduce the impact of negative emotions using Padua-Gabriel´s technique (2010). People rated the negative impact of an emotion on eight consecutive days and in an unexpected follow-up 60 days later. We predicted: (1) lower negative ratings for six vs. three attributes. (2) Reductions in negativity still active after two months.

Method

Participants

Thirty undergraduates, their ages ranged from 18 and 21 years. They were randomly assigned to two groups, $\mathbf{G_1}$ (six attributes) and $\mathbf{G_2}$ (three attributes).

Materials and design

The first and eighth sessions were conducted in our laboratory. Design was a mixed factorial 2 *x* 2. *Group* (6 vs. 3 attributes) varied between-participant. *Session* (1-9) varied within-participant.

Procedure

We asked participants to think of a negative episode in their lives, instructing them to imagine it had six (three) attributes: weight, sound, texture, colour, location in the body, and odour. They used a 10-point scale to rate each dimension daily, performing sessions 2-7 at home when they were relaxed in order to re-think their emotion for about five minutes and
then rated it. They sent daily ratings via internet. Two months later, we run an unexpected follow-up.

Results

Results (all p´s < .001) for $\mathbf{G_1}$ and $\mathbf{G_2}$ show: (1) Steady declines in ratings in sessions 1-8. (2) A further dip in the follow-up. (3) Lower ratings for $\mathbf{G_1}$ than $\mathbf{G_2}$ were indicated by an ANOVA and a linear regression. (4) That $\mathbf{G_1} > \mathbf{G_2}$ was even more evident when we compared them only on the same three attributes (weight, sound and texture).

Discussion

Padua-Gabriel´s (2010) technique is efficient for various reasons: (1) Experimenters do not need extensive training to conduct it; it works out after one single session. (2) Participants learn the technique just as swiftly. (3) The advantage of imagining putative physical dimensions is that they assist people to deal with emotions more effectively by providing tangible "handles" that reduce their severity more easily than when trying to manage the entire negative emotion. (4) We validated ARP´s main prediction: Using six dimensions was more efficient for reducing the arousal caused by a negative emotion than three.

Disclosure.DisclosureBlock:

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.15/III36

Topic: F.01. Human Cognition and Behavior

Title: Activation in posterior parietal cortex correlates with subjective familiarity: Evidence from retrospective confidence ratings and false alarms

Authors: *J. L. VINCENT;
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Abstract: Successful recognition memory decisions depend on mnemonic and decision making processes that are computed by multiple, distributed brain areas. In particular, recent neuroimaging studies have consistently associated parietal cortex with episodic memory retrieval. However, little is known about what computations these areas perform or how these areas are functionally connected. Here, I collected behavioral and functional MRI data from humans (N = 32) during the performance of an old-new recognition memory task with retrospective confidence judgments. Successful recognition memory was associated with activation in multiple regions including anterior, dorsolateral, and medial prefrontal, medial and lateral temporal, retrosplenial and posterior cingulate, as well as lateral parietal cortex. Analyses of resting state functional connectivity demonstrated that these brain regions were organized into two distinct networks, and each network was associated with a distinct region in parietal cortex. The hippocampal-cortical network included the medial temporal lobe, posterior midline structures, and the ventral posterior inferior parietal lobule. The frontoparietal network included lateral prefrontal cortex and intraparietal sulcus. The parietal component of the hippocampal-cortical network was most active during old vs. new decisions, did not differentiate hits from false alarms, and was differentially active during low confidence old and new judgments. In contrast, while the parietal component of the frontoparietal network was robustly activated by hits vs. correct rejections, it was not differentially active during either false alarms vs. correct rejections or low confidence old vs. new judgments. Thus, two distinct regions in parietal cortex can be distinguished by their relative connectivity to the medial temporal lobe vs. lateral prefrontal cortex and their responses during uncertain old judgments and errors. These results demonstrate that ventral posterior inferior parietal lobule is correlated with the subjective perception of familiarity. More generally, these observations suggest a dual network model of recognition memory decision making.

Disclosures: J.L. Vincent: None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.16/III37

Topic: F.01. Human Cognition and Behavior

Title: Dynamic changes in interregional functional connectivity strength during autobiographical memory retrieval

Authors: *C. INMAN[1], G. A. JAMES[2], C. CAMPANELLA[1], T. PATHMAN[3], P. BAUER[1], S. HAMANN[1];
[1]Psychology Dept., Emory Univ., Decatur, GA; [2]Univ. of Arkansas for Med. Sci., LITTLE ROCK, AR; [3]Univ. of North Carolina at Greensboro, Greensboro, NC

Abstract: The brain is comprised of dynamic networks of functional regions that interact with one another to execute various processing demands. Autobiographical memory (AM) involves the orchestration of multiple cognitive processes that evolve over time, including memory access and subsequent elaboration. Previous neuroimaging studies have contrasted memory access and elaboration processes in terms of regional brain activation and connectivity within coordinated multi-region networks rather than between specific regions like the hippocampus and mPFC. The purpose of the present study was to determine the changes in interregional connectivity strength across AM retrieval processes to understand network level mechanisms of AM retrieval and test theoretical accounts of dynamic AM retrieval processes. We predicted that dynamic (time-varying) connections would reflect early memory-access related connectivity between hippocampal and medial PFC regions and a separate set of later, elaboration-related connections between lateral frontal working memory regions and parietal/occipital visual imagery regions. In contrast, stable, time-invariant connections would reflect local networks and frontoparietal control networks that are hypothesized to be active throughout AM retrieval. Healthy adults generated AMs from neutral cue words in a pre-scan session and were later cued to retrieve the AMs for 16 seconds while being scanned with fMRI. We used a moving-window graph theory analysis to examine dynamic changes in the strength of connectivity among regions involved in AM retrieval. Stable connections across AM retrieval included established fronto-parietal control networks and neighboring regions involved in similar functions throughout retrieval. Bilateral connectivity between the amygdala and hippocampus sustained its strength throughout memory retrieval, possibly reflecting ongoing construction and mnemonic binding processes. As predicted, connectivity strength between the hippocampus and medial prefrontal and parietal cortex increased during early access processes. Connectivity strength between precuneus, inferior parietal lobule, and primary visual areas increased later in retrieval, consistent with visual imagery and sensory reactivation processes involved in elaboration. These changes in interregional connectivity strength are consistent with models of AM retrieval that predict dynamic coordination of brain regions supporting search, access, self-reference, episodic reconstruction, and elaboration.

Disclosures: C. Inman: None. **G.A. James:** None. **C. Campanella:** None. **T. Pathman:** None. **P. Bauer:** None. **S. Hamann:** None.

Poster

572. Human Long-Term Memory: Retrieval

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.17/III38

Topic: F.01. Human Cognition and Behavior

Support: DFG, SFB 779

neurodapt!

Title: Retrieval success effects in the nucleus accumbens are coded in early local field potentials and neural theta oscillations: Evidence from human intracranial recordings

Authors: *E. M. BAUCH[1], T. ZAEHLE[2,3], H. HINRICHS[2], F. C. SCHMITT[2], J. VOGES[2], H.-J. HEINZE[2,3], N. BUNZECK[1];
[1]Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Departments of Neurol. and Stereotactic Neurosurg., Otto-von-Guericke Univ., Magdeburg, Germany; [3]Dept. of Behavioral Neurol., Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: Recent functional magnetic resonance imaging studies have highlighted the involvement of the nucleus accumbens (NAcc) in declarative memory retrieval. More specifically, the correct discrimination between studied old and unstudied new items was shown to be associated with enhanced NAcc activations and this has been labeled ‘retrieval success’ effect. Although these findings accord with different memory models, the precise temporal and oscillatory dynamics of the NAcc in memory retrieval remain unclear. Here, we addressed this issue by recording intracranial electroencephalography (iEEG) in human epilepsy patients during a simple old/new recognition test. In a first phase, patients incidentally encoded photographs of scenes in a previously established visual oddball paradigm. After a short delay, memory was tested by presenting the old scenes together with new distractor images and patients were asked to indicate their old/new status by button presses. As a main finding, we can show that differences in local field potentials between correctly classified old (i.e., studied) and new (i.e., unstudied) images emerged already at ~100 ms after stimulus onset and endured for more than 300 ms. Moreover, time-frequency analyses revealed a theta (4-8 Hz) power decrease for old relatively to new items. Together, our findings give new insights into the neural mechanisms of memory retrieval in the NAcc. They are in line with the notion that the NAcc receives memory signals from interconnected brain regions, such as the medial temporal lobe and prefrontal cortex, and that theta oscillations may serve as a mechanism to bind these distributed neural assemblies.

Disclosures: E.M. Bauch: None. **T. Zaehle:** None. **H. Hinrichs:** None. **F.C. Schmitt:** None. **J. Voges:** None. **H. Heinze:** None. **N. Bunzeck:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.18/III39

Topic: F.01. Human Cognition and Behavior

Support: Washington University McDonnell Center for Systems Neuroscience

NSF grant DGE-1143954

Title: Parahippocampal and retrosplenial cortex are more active during remembering than during episodic future thinking

Authors: *A. W. GILMORE, S. M. NELSON, K. B. MCDERMOTT;
Psychology, Washington Univ., Saint Louis, MO

Abstract: Recent neuroimaging studies have demonstrated that both remembering events from the past and imagining events in the future activate a common set of regions within the human brain's default network (Schacter et al., 2012). While researchers have often found regions exhibiting greater activity for imagined future events relative to remembered past events (e.g., Szpunar et al, 2007), the reverse pattern (i.e., Remember > Imagine) has not been consistently observed. A number of hypotheses (Addis et al., 2007; Szpunar et al., 2007) suggest that this is due to the increased processing demands present during future simulation when combining details into a novel, coherent narrative. A reasonable alternative is that studies have simply failed to isolate regions showing greater activity for remembered past events. This subfield of cognitive neuroscience is still in its relative infancy, having come to the fore a little over half a decade ago, and thus the number of studies that have been conducted so far is relatively small. In this study we used functional magnetic resonance imaging (fMRI) to attempt to locate regions that show sensitivity to remembered past events by asking participants to remember events from their past, imagine events that might occur in their future, or imagine events involving a familiar other. Across 3 separate experiments we found regions in paraphippocampal and retrosplenial cortex that show greater activity when remembering past events than in other experimental conditions. By using both standard rapid event-related and catch trial fMRI designs (Ollinger et al., 2001), we found that this sensitivity persists when examining task trials as a whole, or when controlling for possible differences in trial-by-trial task orientation signals in the BOLD response. These regions have previously been implicated in mental simulation, and sit adjacent to, but outside of, the default network. They correspond to locations that are thought to be involved in processing contextual information (Bar, 2009). We hypothesize from these results that a signature of past experience is, at least in part, based on the amount of contextual information associated with a given event during episodic simulation.

Disclosures: A.W. Gilmore: None. **S.M. Nelson:** None. **K.B. McDermott:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.19/III40

Topic: F.01. Human Cognition and Behavior

Support: Israeli Science Foundation (MT)

Foundation Adelis (MT)

Human Frontier Science Program long-term fellowship (SR)

NIH grant MH55687 (to M. Kahana)

Title: Associative retrieval mediates free recall of unrelated words

Authors: M. KATKOV[1], S. ROMANI[2,3], *M. V. TSODYKS[1];
[1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Ctr. for Theoretical Neurosci., Columbia Univ., New York, NY; [3]Donders Ctr. for Neurosci., Radboud Univ., Nijmegen, Netherlands

Abstract: Human subjects successfully recognize up to 10,000 items after a brief exposure. Yet retrieving information from memory can be challenging if no specific cues are provided. In the classical free-recall paradigm, where participants are prompted to recall a list of previously presented words, the number of recalled items appears to exhibit sub-linear scaling with the number of remembered items. Notably, more words can be recalled with appropriate cues, indicating that the difficulty to recall is not caused by true forgetting. Similar results were obtained for a different paradigm where words are recalled based on graphemical cues (words beginning from letter ‘a’, ‘b’, etc.).
Here we propose a new model of free recall, based on neural network model of associative retrieval. Memorized items are stored in the network by strengthening connections between specific groups of neurons, resulting in stable activity patterns (attractors) that encode different items. Retrieval of memory is mediated by the activation of the corresponding attractor with each retrieved item triggering the next item. A sequence of attractors visited by the network activity represents items retrieved in a given trial. The dynamics of the retrieval is defined by the combination of four effects: the overlap of neuronal populations encoding presented items (semantic similarity), strengthened representation of first presented item (primacy), additional similarity acquired by the newly presented item and one of the previous items (contiguity) and,

finally, short-term memory for one of the last presented items (recency). The model also predicts that items are easier or more difficult to recall, i.e. their overall recall probability is correspondingly higher/lower, depending on how large their neuronal representation is.
We found that the model can account for the observed overall distribution of number of recalled items across trials, distribution of recall probabilities across items, serial position curve (recall probability vs. position of an item in the list) and distribution of recalled chain length, where a chain is defined as a sequence of items recalled either in the presented (positive length) or reverse (negative length) order. A more subtle, counterintuitive, prediction of the model is confirmed by data analysis (data courtesy of M. Kahana). For both the model and the data, the number of recalled words decreases, on average, with their mean recall probability, while it is roughly independent of the mean recall probability of presented words. The results support our hypothesis that recall of information from long-term memory is mediated by associative transitions between memory items.

Disclosures: M. Katkov: None. **S. Romani:** None. **M.V. Tsodyks:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.20/III41

Topic: F.01. Human Cognition and Behavior

Support: NSC101-2410-H-008-034-MY3

Title: Source memory performance is modulated by transcranial direct-current stimulation over the left posterior parietal cortex

Authors: *C. LO, N. CHEN, N. G. MUGGLETON, C. JUAN, S. CHENG;
Inst. of Cognitive Neurosci., Inst. of Cognitive Neuroscience, Natl. Central Univ., Jhongli, Taiwan

Abstract: In functional neuroimaging studies, it has been consistently found that the left posterior parietal cortex (LPPC) shows retrieval-related activation during memory tasks. However, the specific role of the LPPC during memory retrieval remains unclear. In particular, the correlational nature of neuroimaging studies makes it difficult to establish causal relationship between LPPC and memory retrieval. In the present study, transcranial direct-current stimulation (tDCS) was used to address this issue. Previous studies on motor cortex show that different polarities of tDCS (anodal and cathodal) can facilitate and inhibit neural excitability respectively. By manipulating the polarities of tDCS over the P3 electrode site (10-20 system), we predicted

that memory performance would be increased by anodal stimulation, whereas cathodal stimulation would decrease memory performance. Twenty-four subjects were divided into two groups (12 anodal and 12 cathodal). Each participant was asked to come to the lab three times where source memory tasks were presented during each visit. All subjects received stimulation over P3, sham, and right primary motor cortex (M1) as the control site each on different visits. On-line tDCS was delivered throughout the entire test phase. The old/new recognition performance showed no significant differences between stimulation over P3, sham, and M1 in both anodal and cathodal groups. However, compared with the sham condition, we found that source memory accuracy was decreased significantly in the cathodal group when subjects received stimulation over P3, whereas no such effect was found in the anodal group. This result shows that the effects of cathodal stimulation are specific to source memory which engages the recollection process, and not familiarity. Although it remains unclear why there was no effect during anodal stimulation, the data shows source memory accuracy was decreased by cathodal stimulation over P3, providing evidence for the causal relationship between LPPC activity and episodic memory retrieval.

Disclosures: C. Lo: None. **N. Chen:** None. **N.G. Muggleton:** None. **C. Juan:** None. **S. Cheng:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.21/III42

Topic: F.01. Human Cognition and Behavior

Support: Universidad de Guanajuato

CONACYT Grant 60645

Title: Brain Derived Neurotrophic Factor is correlated with memory processes in healthy women

Authors: *L. C. MORADO[1], J. RAMIREZ-EMILIANO[2], M. S. SOLIS-ORTIZ[2];
[1]Med. Sci., Univ. of Guanajuato, León, Mexico; [2]Med. Sci., Univ. of Guanajuayo, Leon, Mexico

Abstract: The Brain Derived Neurotrophic Factor (BDNF) is considered as a biomarker of cognition and plays an important role in memory and learning and neuronal plasticity. High levels of BDNF have been found in the ovulatory and luteal phases of the menstrual cycle in young women and a decrease in the postmenopause. However if the BDNF correlates with memory in women is not well known. The aim of the present study was to correlate the levels of

BDNF with memory tasks in healthy women.
The levels of BDNF in platelets were obtained in 31 premenopausal women, 11 in the ovulatory phase, 10 in the luteal phase and 10 in the menstrual phase, and were compared with 15 postmenopausal women. The scores on 4 tasks measuring working memory, spatial memory, verbal memory, visual memory were obtained for each participant. The number of correct responses, errors, reaction time, omission, and learning of number sequences forward and backward were computed and compared between premenopausal and postmenopausal women. The scores obtained were correlated with the levels of BDNF.
BDNF levels did not differ between premenopausal women and postmenopausal women. BDNF levels were negatively correlated with the number of errors in the working memory task ($r = -0.38$, $p < 0.05$) and with the number of words learned from the verbal memory task ($r = -0.36$, $p = 0.05$) in postmenopausal women.
These findings suggested an association between memory processes, particularly in working memory and verbal memory, and levels of BDNF in premenopausal women probably related to hormonal status.

Disclosures: L.C. Morado: None. **J. Ramirez-Emiliano:** None. **M.S. Solis-Ortiz:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.22/III43

Topic: F.01. Human Cognition and Behavior

Title: Relationship between physiological and clinical measures of prospective memory

Authors: *S. A. RASKIN, N. KAUR, C. PEDRO;
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Abstract: Prospective memory (PM) requires the ability to form and later realize intentions that are delayed over time (Einstein & McDaniel, 1990). The purpose of this experiment was to examine the underlying brain activity related to PM using an event-related potential paradigm (West & Ross-Munroe, 2002) compared to a clinical measure, the Memory for Intentions Screening Test (MIST) (Raskin, Buckheit, & Sherrod, 2011) in both healthy adults (HA) and individuals with traumatic brain injury (TBI). Results revealed that individuals with TBI performed significantly worse than HA on all variables of the MIST and also showed reduced amplitude on ERPs that have been associated with intention formation and intention retrieval when compared to HA. In addition, total score on the MIST was related to variables associated

with attention retrieval. Overall, these findings suggest that individuals with TBI have deficits in PM compared to HA and that the MIST may be a valid measure of underlying brain processes in PM.

Disclosures: S.A. Raskin: Other; Author of clinical measure. **N. Kaur:** None. **C. Pedro:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.23/III44

Topic: F.01. Human Cognition and Behavior

Support: BrainGain Smart Mix Programme of the Netherlands Ministry of Economic Affairs and the Netherlands Ministry of Education, Culture and Science

The Netherlands Organization for Scientific Research (NWO) VICI grant number: 453 09-002

‘‘The healthy brain’’ funded by the Netherlands Initiative Brain and Cognition under grant number 056-14-01

The Dutch Technology Foundation STW, applied science division of NWO and the Technology Program of the Ministry of Economic Affairs, and the University of Utrecht, grant UGT7685.

Title: Gamma bursts surf alpha waves: An ECoG study

Authors: *A. BAHRAMISHARIF[1], M. A. J. VAN GERVEN[1], E. J. AARNOUTSE[2], M. R. MERCIER[3], T. H. SCHWARTZ[4], J. J. FOXE[3], N. F. RAMSEY[2], O. JENSEN[1];
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Abstract: Traveling waves reflected by consistent phase differences across spatially adjacent electrodes have been reported in both human and animal intracranial recordings. While waves in the alpha band (8-13 Hz) have been shown to travel over space in terms of consistent phase differences, it is unknown how this phenomenon relates to neuronal activity in other frequency bands. Recent work has demonstrated that gamma amplitude (30-100 Hz) is coupled to the phase of ongoing alpha oscillations. This raises the question of whether gamma activity is also linked to the phase of alpha oscillations traveling over cortex. We analyzed resting state

electrocorticography (ECoG) data recorded from four epileptic patients. Our first observation was that alpha power dominated the spectral content in most ECoG electrodes. We found examples in each subject where traveling waves were observed in lines of neighboring electrodes. The traveling velocity was in the range of 0.7 to 2.3 m/s. By calculating the coherence in different frequency bands, we found that the traveling waves were specific to the alpha band. A cross-frequency analysis taking all frequency combinations into account, revealed a strong modulation of gamma power by alpha phase. Modulations were not robust in other bands. We further showed that the gamma bursts were phase-locked to traveling alpha waves occurring at the troughs. As a result the gamma activity 'surfs' the alpha waves. Given that alpha activity has been proposed to modulate neuronal processing reflected in the gamma band, we suggest that alpha waves are involved in coordinating neuronal processing in both space and time. In future work it would be of great interest to investigate if this phenomenon reflects behavior and cognitive processing.

Disclosures: A. Bahramisharif: None. **M.A.J. van Gerven:** None. **E.J. Aarnoutse:** None. **M.R. Mercier:** None. **T.H. Schwartz:** None. **J.J. Foxe:** None. **N.F. Ramsey:** None. **O. Jensen:** None.

Poster

572. **Human Long-Term Memory: Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 572.24/III45

Topic: F.01. Human Cognition and Behavior

Support: NWO VICI Grant #453-09-002

Title: Local entrainment of alpha oscillations by visual stimuli phasically modulates perception

Authors: *E. SPAAK, F. P. DE LANGE, O. JENSEN;
Radboud Univ. Nijmegen, Donders Inst. For Brain, Cognition, and Behaviour, Nijmegen, Netherlands

Abstract: Prestimulus oscillatory neural activity greatly influences the detection of near-threshold stimuli. In particular, the amplitude and phase of ongoing alpha oscillations has been shown to predict whether near-threshold stimuli are detected or not. To make a causal claim about the mechanistic role of oscillatory activity, one needs to be able to manipulate the oscillations directly, independently of any cognitive or attentional instruction. Recently, several authors have attempted such manipulations through transcranial magnetic or current stimulation, and noninvasively through the presentation of periodic stimuli. Here we present compelling

evidence that rhythmic visual stimulation entrains ongoing alpha oscillations in human visual cortex, in a spatially specific manner. We presented rhythmic stimuli to one visual hemifield, and arhythmic stimuli to the other. We find a periodic pattern in detection performance of a subsequent near-threshold target only for the hemifield that was rhythmically stimulated, and not for the other hemifield. This periodic pattern lasts for at least three oscillatory cycles. The magnetoencephalogram (MEG) was measured throughout the experiment. We find strongly lateralized alpha activity in the MEG, phase-locked to the rhythmic stimulation, and outlasting the stimulation by several cycles. Using a beamformer apporach, we localized the sources of the entrained alpha activity to primary visual cortex. Importantly, the degree of entrainment quantified from the MEG data predicted the behavior: subjects with a stronger alpha entrainment were also subjects with a stronger phasic modulation of detection performance in the entrained hemifield. Taken together, these findings argue for a cortically focal entrainment of ongoing alpha oscillations by visual stimulation, with concomitant consequences for perception. The findings support the notion that oscillatory brain activity in the alpha band provides a causal mechanism for temporally organizing visual perception.

Disclosures: E. Spaak: None. **F.P. de Lange:** None. **O. Jensen:** None.

573**Poster**

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.01/III46

Topic: F.01. Human Cognition and Behavior

Support: AFOSR Grant FA9550-12-1-0369

Title: Computational model of exponentially decaying persistent firing for encoding stimulus history

Authors: *Z. TIGANJ, K. SHANKAR, M. HOWARD;
Ctr. For Memory and Brain, Boston Univ., Boston, MA

Abstract: Persistent firing, observed in variety of brain regions, is believed to be important for working memory. However, persistent firing with stable firing frequency does not carry any information about the timing of stimulus presentation and does not account for some well-known characteristics of memory such as scale-invariance and forgetting. It has been shown that persistent firing with exponentially decaying firing rate can be an essential building block of working memory [1]. In order to represent several minutes of the recent stimulus history the largest time constant should also be several minutes. Through a computational study we

demonstrate here that exponentially decaying persistent firing with a range of time constants up to minutes is biologically plausible.
We show that calcium controlled cation current and interplay between calcium pumps and calcium inward currents could account for theoretically arbitrarily long time constants. We first assume that the stimulus presentation results in large calcium influx. After the stimulus has ended, the calcium controlled cation current can be sufficiently large to iteratively depolarize the cell to the firing threshold. If the amount of calcium that leaves the cell during interspike intervals is greater than the amount that enters the cell during spikes the overall calcium concentration will decay. Consequently the calcium controlled cation current will also decay and the time it takes to depolarize the cell to the firing threshold will increase from spike to spike, resulting in a decay of the firing rate as well. We show that this decay can be exponential with a time constant several orders of magnitude larger than any intrinsic time constant of the cell. Moreover, we show that these time constants can be controlled externally with dendritic inhibition allowing the system to account for various temporal, ordinal, and spatial representations.
1. Shankar, KH, and Howard, MW: A scale-invariant internal representation of time. Neural Computation 2012, 24:134-193.

Disclosures: Z. Tiganj: None. **K. Shankar:** None. **M. Howard:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.02/JJJ1

Topic: F.01. Human Cognition and Behavior

Title: Increasing functional connectivity with cognitive load

Authors: *L. AHONEN[1,2], M. HUOTILAINEN[2];
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Abstract: Studying working memory (WM) with computerized classical cognitive tests is an efficient way to investigate functional activation patterns in brain with different cognitive load levels. The present study investigated neural activity using magnetoenchepalography (MEG) in N-back paradigm. N-back is a task that according to previous research activates large number of WM related brain areas. There are also preliminary results in functional magnetic resonance imaging (fMRI) suggesting that lateral and other inter-areal connectivity between WM associated areas increase during higher cognitive loads. N-back provides precise way to control

cognitive load and working memory related activation. We conducted connectivity analysis based on Granger causality to test functional relations between brain regions that have been identified to be task related networks. We focused on load-dependent changes in event-related activity originating from key brain areas in frontal and parietal regions and tested the connectivity in different task conditions. We found increased fronto-parietal and lateral connections with more difficult task conditions. The connectivity was increasing in the direction from frontal areas to parietal areas and between the hemispheres. These results are in line with the previous literature as the number the active brain regions goes up, the connectivity between the related areas become more prominent. These results reveal the functional networking between brain regions during updating and maintaining task dependent information. The connections from frontal regions might generalize to other cognitively demanding tasks.

Disclosures: L. Ahonen: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Valio Ltd.. **M. Huotilainen:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.03/JJJ2

Topic: F.01. Human Cognition and Behavior

Support: MRC

Title: Oscillatory visual activity associated with preparatory attention and working memory maintenance

Authors: *B. CRITTENDEN, M. P. NOONAN, N. ADAMIAN, M. STOKES;
OHBA, Oxford, United Kingdom

Abstract: During the delay period of working memory tasks it can be difficult to disentangle working memory maintenance activity from preparatory activity in anticipation of the upcoming memory probe stimulus. This study was designed to decouple the contribution of these two processes by investigating changes in the time-frequency spectrum during the delay period by orthogonally manipulating the working memory load and need for top-down preparatory attention.
Six participants each took part in 4 one-hour EEG sessions. Participants were asked to attend to either 1, 2 or 4 simultaneously presented memory stimuli (small circular gratings) that appeared on screen for 250ms. Participants were then required to retain the orientation of these gratings over a 2.5s delay period as best they could. Following the delay, a memory probe stimulus

appeared in one of the previously to-be-remembered stimuli locations. Participants were asked to indicate whether the memory probe was rotated clockwise or anticlockwise relative to the memory item that was previously in that location. The difficulty of the task was manipulated in two ways: the contrast of the memory probe was either high or low, with contrast fixed within blocks; in addition the rotation of the probe could be either 45°, 23°, 11° or 6°, with rotation size varying on a trial-by-trial basis.
EEG was recorded over 64-scalp electrodes using a synamps amplifier. Muscle and eye movements were also recorded and used to reject trials contaminated with saccadic and other movement related artefacts. Each session was high (45Hz) and low-pass (0.05Hz) filtered and aligned with the behavioural responses and trial-type triggers.
Looking across posterior electrodes, we found a significant difference between set size 4 and set size 1 in the alpha frequency (8-12Hz) range when participants were expecting a low-contrast memory-probe stimulus. There was, however, no set size effect when participants were expecting a high-contrast memory probe stimulus.

Disclosures: B. Crittenden: None. **M.P. Noonan:** None. **N. Adamian:** None. **M. Stokes:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.04/JJJ3

Topic: F.01. Human Cognition and Behavior

Support: DARPA-10-55-Open-BAA-FP-198

Title: EnygmaGym: Static versus dynamic visual computer based training results in discrete morphometric changes

Authors: *K. FUJIMOTO[1], A. KATZMAN[2], S. N. NIOGI[3], B. B. ALLEN[4], M. FASO[5], U. RAJASHEKAR[5], I. TSERETOPOULOS[5], J. MARUTA[5], J. GHAJAR[5], B. E. KOSOFSKY[2,4]; [1]Neurol., [2]Pediatrics, Weil Cornell Med. Col., New York, NY; [3]Diagnos. Radiology, [4]Neurol., NewYork-Presbyterian Hosp., New York, NY; [5]Brain Trauma Fndn., New York, NY

Abstract: Background:
We utilized MR-based morphometry to identify the regional specificity of structural changes in the brains of volunteers with one of two computer-based visual exercises: a dynamic exercise which requires predictive timing, and a static exercise which involves working memory.
Methods:

22 healthy volunteers who participated in the study were randomly divided into two training groups: group 1 with 12 volunteers (mean age = 23.3 ± 2.1) and group 2 with 10 volunteers (mean age = 22.6 ± 2.1). Each volunteer played the assigned exercise for at least three ten-minute sessions each day, five days a week over eight weeks. Three T1-weighted images were acquired on each subject before (0 weeks), during (4 weeks) and after (8 weeks) the training. All images were processed with FreeSurfer for cortical parcellation and subcortical segmentation, followed by a within-subject robust analysis of longitudinal morphometric changes. To assess the significance of changes within each training group, a two-tailed paired t-test was performed on each segmented subcortical and cortical volume between the first and last scans. Investigators remained blinded to the training groups.

Results:

We observed global and region-specific cortical thinning in group 1 after the eight weeks of training. A two-tailed paired t-test showed that the whole-brain cortical grey volume of group 1 significantly decreased (total cortical grey p=0.026, left cortical grey p=0.074, and right cortical grey p=0.008). Regional reductions in cortical volume for group 1 are shown in the accompanying table. However, the cortical grey volume for group 2 remained unchanged after the eight weeks of training.

Conclusion:

In a blinded analysis of cortical morphometric changes induced by an eight-week intensive training paradigm, we observed a differential effect of static versus dynamic visual exercises. We found a decrease in cortical volume in one group but not the other. Interestingly, regional reductions in cortical volume were more driven by changes in the right hemisphere, and were localized to regions known to subserve attention and predictive timing.

Brain Areas with Significant Reductions in Cortical Volume ($p < 0.05$)

	Left Hemisphere	Right Hemisphere
A) Attention System Network:		
1) Cingulate Gyrus		
Caudal anterior cingulate		0.017
2) Inferior Parietal Lobule		
Inferior parietal cortex	0.018	
Supramarginal gyrus	0.005	0.023

B) Frontal Cortex:		
Rostral middle frontal		0.017
Superior frontal		0.017
Caudal middle frontal		0.027
C) Other Regions:		
Superior parietal	0.011	0.017
Cuneus		0.011

Disclosures: K. Fujimoto: None. **A. Katzman:** None. **S.N. Niogi:** None. **B.B. Allen:** None. **M. Faso:** None. **U. Rajashekar:** None. **I. Tseretopoulos:** None. **J. Maruta:** None. **J. Ghajar:** None. **B.E. Kosofsky:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.05/JJJ4

Topic: F.01. Human Cognition and Behavior

Support: The 111 Project (B07008) of the Ministry of Education of China

National Natural Science Foundation of China (31100807)

The Research Fund for the Dectoral Program of Higher Education (20110003120001)

Title: Interaction effects of BDNF and COMT on brain resting-state regional homogeneity

Authors: *W. CHEN, C. CHEN, B. ZHU, X. LEI, Q. DONG;
State Key Lab. of Cognitive Neurosci. and Learning, State Key Lab. of Cognitive Neurosci. and Learning, Beijing, China

Abstract: Introduction: Catechol-O- methyltransferase (COMT) and brain-derived neurotrophic factor (BDNF) genes have been proved to interactively influence working memory (WM) as well as brain activation during WM tasks. However, whether the two genes have interactive effects on resting-state activities of brain and whether these spontaneous activation influence WM are still unknown.
Methods: In this study, three hundred and sixteen healthy Han Chinese subjects were scanned during the resting state, genotyped on COMT rs4680 (169 COMT-Val/Val Val/Val, 139 COMT-Met), and BDNF Val66Met (85 BDNF-Val/Val, 231 BDNF-Met, and finished a 2-back WM task. Resting-state fMRI data were preprocessed and calculated regional homogeneity (ReHo) using DPARSF package (www.restfmri.net). Genetic effects on ReHo were investigated using SPM. For regions that showed significant COMT-BDNF interaction, mean ReHo was extracted. Correlations between mean ReHo and WM performance were tested.
Results: Significant interactive effects of BDNF and COMT were observed in Default Mode Network (DMN) and WM related brain areas, including left posterior cingulate cortex, bilateral precuneus, right middle frontal gyrus, right middle temporal gyrus and right superior parietal lobule (Fig. 1a). Further simple analyses show that COMT Val/Val have higher ReHo than COMT Met carriers in BDNF Met carriers and have opposing effect in BDNF Val homozygote only in right superior parietal lobule, which was reverse in other areas (Fig. 2b). What's more, WM performance was positively correlated with the mean ReHo of right superior parietal lobule while negatively correlated with that of right middle frontal gyrus.
Conclusions: The findings support the hypothesis that COMT and BDNF polymorphisms influences brain spontaneous ReHo, which can further predict WM performance, suggesting that resting-state ReHo can be a reliable endophenotype that bridges genetic variation to behavior.

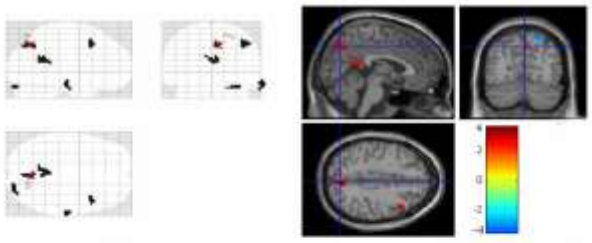

Figure 1a Interaction effects of COMT and BDNF on ReHo of the whole brain. The coordinates is [3.42, -71.40, 43.04].

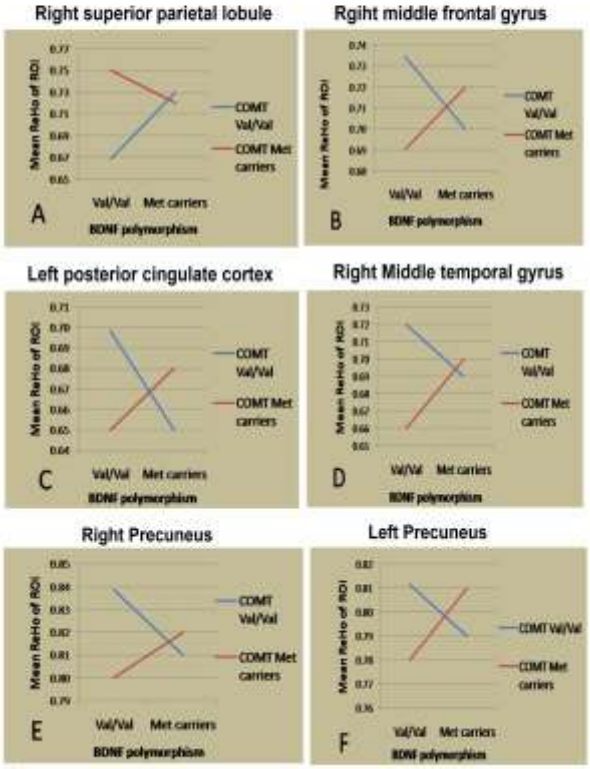

Figure 1b Simple effects of COMT and BDNF on mean ReHo in significant ROIs.

Disclosures: W. Chen: None. **C. Chen:** None. **B. Zhu:** None. **X. Lei:** None. **Q. Dong:** None.

Poster

573. Working Memory and Executive Function III

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.06/JJJ5

Topic: F.01. Human Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft (DFG)

Title: Neural basis of object-based shifting of attention in working memory

Authors: *B. PETERS[1], B. RAHM[2], J. KAISER[1], C. BLEDOWSKI[1];
[1]Inst. of Med. Psychology, Goethe-University, Frankfurt Am Main, Germany; [2]Med. Psychology and Med. Sociology, Univ. Med. Ctr. Mainz, Mainz, Germany

Abstract: Working memory (WM) enables the retention of a limited number of items for a short period of time. In addition, many situations require that a subset of WM contents be transiently prioritized for processing by focusing attention on them. Current models of WM suggest that the same attentional mechanisms that are known from perception also operate in WM. In vision, shifts of attention between spatial positions within the boundaries of one object are performed faster than shifts between positions located on different objects. This within-object benefit can be explained by an automatic spread of attention within perceived object boundaries in visual cortex.
Hypothesizing the same attentional mechanisms in WM as in perception, we tested whether the within-object benefit can be observed, both on the behavioral and neural level, when subjects focus attention on spatial positions that are no longer physically present but represented in WM. 20 healthy subjects were presented two objects each containing two highlighted spatial positions. They had to memorize all four spatial positions. Attentional shifts in WM were faster for spatial positions located on the same object compared with equidistant positions on separate objects. Shifting attention in WM thus showed a within-object benefit comparable to the effect observed in a perceptual version of the same task. This behavioral benefit was associated with increased BOLD activity in posterior parietal cortex that could not be explained by differences in eye movements between conditions. Moreover, analysis of retinotopic visual cortex revealed that the automatic spread of attention within object boundaries was present also for information held in WM. Specifically, when attention was shifted to a memorized position, activity in early visual areas was enhanced at the retinotopic location corresponding to the second position co-located on the same object compared to equidistant positions located on the other object.
These results extend the previous suggestion of shared mechanisms of spatial attention in perception and WM by demonstrating that this notion also holds for object-based attention. Attentional selection of a memorized position leads to a neural co-activation of another memorized position within the same object. This suggests that when object-like representations are held in WM, attentional selection includes activation of the complete object, thus accounting for the within-object benefit.

Disclosures: B. Peters: None. **C. Bledowski:** None. **J. Kaiser:** None. **B. Rahm:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.07/JJJ6

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01DA02463 (PI: Fiez)

Title: Nicotine-deprived smokers' WM capacity differences in strategy use and recruitment of brain regions linked to goal maintenance during a WM task

Authors: *A. S. WEIGARD, S. WILSON, C. HUANG-POLLOCK;
The Pennsylvania State Univ., University Park, PA

Abstract: Working Memory (WM) performance in smokers is degraded by nicotine deprivation, but how individual differences in WM capacity (WMC) affect task strategies in this condition is unclear. On a WM recognition task, individuals can use evidence of stimulus familiarity or evidence from the recollection of the stimulus representation in WM (Oberauer, 2005). While a familiarity strategy relies on simple recognition, recollection requires active maintenance of WM items and task goals, suggesting that high WMC individuals, who display increased goal maintenance (Unsworth & Engle, 2007), may primarily use a recollection strategy while low WMC individuals may rely on familiarity. The current study investigates this possibility, with predictions that 1) low WMC, but not high WMC, smokers will display a strong relationship between basic recognition speed and WM performance and 2) high WMC smokers' performance will be more strongly associated with activity in brain regions linked to goal-maintenance. A sample of 125 active smokers was split into high and low WMC groups (median split) based on scores on the OSPAN task (Turner & Engle, 1989) completed prior to the experiment. After abstaining from nicotine for 12 hours, participants completed an N-back task during fMRI data acquisition. In each block, participants were presented with 12 letters in a 0-back condition (speed), where they pressed a button with their right index finger when the letter "X" appeared, or in a 3-back condition (WM performance), where they pressed the button if the letter presented matched one presented three items prior. In both conditions, participants pressed a button with their right middle finger for non-targets. RT data from each condition was fit to the diffusion model (Ratcliff & McKoon, 2008), which indexes speed of processing on decision tasks as the parameter of drift rate (v). The low WMC group displayed a strong association between v on the 0-back and v on the 3-back, $r(63) = .44$, $p < .001$, $R^2 = .19$, while high WMC individuals did not, $r(62) = .20$, $p = .12$, $R^2 = .04$, suggesting that for low WMC individuals, but not high WMC individuals, performance on WM recognition tasks was highly related to simple recognition speed. This supports the hypothesis that low WMC smokers primarily use a familiarity strategy. To determine if high WMC individuals' WM performance is more strongly associated with recruitment of brain regions implicated in goal-directed behavior than low-WMC individuals',

regression analyses will be conducted where activity in specific regions of interest, the dorsolateral prefrontal cortex and anterior cingulate, during the 3-back is used to predict *v* on the 3-back in each group.

Disclosures: A.S. Weigard: None. **S. Wilson:** None. **C. Huang-Pollock:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.08/JJJ7

Topic: F.01. Human Cognition and Behavior

Support: NIH grant F32EY022874 to KCB

NIH grant 1R01EY022355 to YX

Title: Decoding visual short-term memory contents in occipital and parietal cortices under distraction

Authors: *K. BETTENCOURT, Y. XU;
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Abstract: Visual short-term memory (VSTM) has been shown to recruit a network of brain regions, including frontal, parietal, and posterior sensory regions. However, results from different types of analyses disagree on where exactly the contents of VSTM are stored. Univariate fMRI analyses have suggested that regions in the parietal lobe, in particular superior intraparietal sulcus (IPS), play a central role in VSTM information storage. In contrast, with the exception of one study, research with fMRI multivariate pattern analysis (MVPA) has shown that the contents of VSTM can only be decoded in early visual areas, and not in areas that show sustained load related univariate delay period activity. Thus, it remains unclear where the contents of VSTM are stored. Furthermore, if information is stored within the pattern of delay period activity in early visual cortex, as suggested by MVPA, then what happens when we are faced with distracting visual information during the delay? Given the ubiquitous presence of distractors in everyday visual perception, any area involved in the storage of VSTM must retain visual information under distraction. Here, in an fMRI study using MVPA, we show that while a particular orientation held in VSTM can be successfully decoded from early visual areas in the absence of distractors, decoding performance significantly dropped when distractors are introduced during the delay period. Importantly, behavioral performance is equally good with and without distractors, showing that the decrease in decoding in early visual areas under

distraction cannot be attributed to poor task performance. In contrast, decoding performance in superior IPS is significantly above chance regardless of whether distractors are present or not. These results indicate that parietal regions likely play a central role in VSTM information storage across a variety of stimulus conditions. In the absence of distraction, early visual areas may be recruited to aid the maintenance of memory representations, likely via a visual rehearsal/mental imagery strategy. However, such contributions are unlikely to be essential for VSTM information storage in the real world where visual distraction is constant.

Disclosures: K. Bettencourt: None. **Y. Xu:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.09/JJJ8

Topic: F.01. Human Cognition and Behavior

Support: NIH 5R90DA033460

NSF DMS-1042134

Title: ACC control of DLPFC interneurons tunes beta/gamma oscillation and inter-areal feedback in a laminar spiking model

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Abstract: Interactions between dorsolateral prefrontal cortex (dlPFC), anterior cingulate (ACC), and sensory association cortices underlie the maintenance and manipulation of cell assemblies activated by previous sensory stimuli. This persistent “delay” activity has been implicated in working memory and cognitive control processes, but the mechanisms by which it contributes to these processes are unknown. We hypothesize that beta and gamma oscillations in interacting dlPFC & ACC control the transition between two dlPFC states: (1) one where stimulus-related activity persists after stimulus offset without modulating sensory cortices, and (2) one where persistent firing is locally coordinated by network oscillations into synchronous subassemblies that feedback and interact with activity in association cortex.
To investigate the cellular and network mechanisms that could support this behavior, we developed a computational spiking neuron model of laminar dlPFC with feedforward inputs from auditory association cortex and feedback from ACC. The dlPFC model included superficial and

deep layers with the following components: (1) a recurrent pyramidal network supporting delay activity, (2) modulatory and fast spiking interneurons, (3) beta- and gamma-generating mechanisms, (4) ACC input to superficial dlPFC and sensory inputs to middle layers. Our results indicate that (1) ACC activation of interneurons can mediate dlPFC network oscillations in the beta- and gamma-frequency ranges and (2) sensory inputs can drive stimulus-related subassemblies that fire at distributed phases of the dlPFC network oscillations. Together, these results suggest ACC may synchronize dlPFC assemblies for effective phase-coded feedback to modulate processing in association cortices. This represents a mechanism by which ACC could switch local dlPFC subassemblies from a passive maintenance to active modulatory mode. Our future studies will investigate the specific contributions of gamma and beta rhythms in different layers of dlPFC & ACC as well as the effects of coordinated feedback from prefrontal cortices on afferent sensory processing. These simulations will facilitate mechanistic theories linking working memory processes to cognitive control and attentional regulation of afferent sensory streams.

Disclosures: J.S. Sherfey: None. **N. Kopell:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.10/JJJ9

Topic: F.01. Human Cognition and Behavior

Support: ACR Investigator Award

Georgetown and Howard Universities Center for Clinical and Translational Research

Title: The association between cognitive performance, somatic perception, and task-related brain activity in fibromyalgia patients and healthy volunteers

Authors: *B. WALITT[1], M. KHATIWADA[2], M. CEKO[3], J. VAN METER[2], R. GRACELY[4]; [1]Medstar Washington Hosp. Ctr., Washington, DC; [2]Georgetown Univ., Washington, DC; [3]NCCAM, NIH, Bethesda, MD; [4]Univ. of North Carolina, Chapel Hill, NC

Abstract: Introduction: Perceived cognitive dysfunction is a common complaint in fibromyalgia (FM). Experimental evidence suggests that FM patients activate cognitive task-related brain areas differently than healthy volunteers (Glass et al. 2011; Seo et al. 2012). This study addresses the mechanisms mediating these differences, in particular how the distressing somatic perceptions of FM (experimental pain, spontaneous pain, fatigue, functional disability, and

cognitive self-appraisal) influence cognitive task performance and task-related brain activity.
Methods: 16 FM and 13 healthy age, gender, and racially matched controls underwent a total of 69 study visits as part of an interventional trial of exercise therapy. No differences were seen in N-Back performance related to the exercise intervention, allowing us to combine all visits into the two groups. At each visit, the participants completed questionnaires measuring somatic and psychological symptoms and performed N-Back testing of working memory while undergoing BOLD fMRI scanning (block design; 0-Back alternating with 2-Back). Group differences in BOLD fMRI activity (2-Back > 0-Back) were examined using GLM flex in SPM. Voxel-wise regression analysis was performed to determine correlations between BOLD activity and 2-Back accuracy or symptom reporting. Pearson correlations were used to examine the correlation between 2-Back accuracy and symptom reporting.
Results: Healthy volunteers had greater task-related and limbic activation compared to controls (FDR $p< 0.05$) despite no statistical difference in 2-Back accuracy (56.4% v 65.8%, $p= 0.2$). Task-related activity correlated with accuracy in healthy volunteers but not in FM. In FM, task-related and limbic activity correlated with the patient's perception of their ability to attend, their verbal memory, and spontaneous pain. No correlations were found between task-related brain activity and nociceptive pain, mental fatigue, or functional disability.
Conclusion: The expected relation between task-related activation and performance is observed in healthy volunteers but is not found in FM. Our results confirm the group difference reported by Seo et al. but unexpectedly does not show any association between accuracy and brain activity in FM. Task-related brain activity does not account well for cognitive performance in FM patients.

Disclosures: B. Walitt: None. **M. Khatiwada:** None. **J. Van Meter:** None. **M. Ceko:** None. **R. Gracely:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.11/JJJ10

Topic: F.01. Human Cognition and Behavior

Title: Acute sleep deprivation shows readily reversible effects on the Multi-Source Interference Task (MSIT)

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Abstract: Sleep loss is a common occurrence and has recently come under scrutiny as the determining cause of many catastrophic events. Previous studies (Harrison & Horne, 2000) have shown that sleep deprivation can have deleterious effects on cognitive performance and decision-making, however the neural correlates of sleep deprivation are not yet fully understood. The Multi-Source interference task (MSIT) combines elements of three different tasks (Stroop task, Eriksen flanker task, and Simon effect) to induce cognitive interference and robust activation of the dorsal anterior cingulate cortex (dACC) and dorso-lateral prefrontal cortex (DLPFC) (Bush et al., 2003). Previous studies have shown inconsistent findings when testing the effects of acute sleep loss on executive function (Drummond et al., 2006; Kilgore et al., 2006; Cain et al., 2011). The aim of this study was two-fold: (1) to determine how sleep deprivation affects executive function using the MSIT and (2) to use functional MRI (fMRI) to identify the neural regions that are preferentially effected during sleep deprivation, using the general linear model (GLM) and multivoxel pattern analysis (MVPA) analyses.

Study volunteers performed the MSIT while undergoing three fMRI sessions. Each session consisted of three MSIT runs. In the 1st session, subjects achieved three nights of normal sleep (greater than 7 hours of sleep per night). During the 2nd session, subjects achieved complete sleep deprivation for at least 24 hours. In the final session, subjects returned after 2 nights of recovery sleep.

Subjects showed delayed reaction times in the neutral condition during sleep deprivation compared to their initial scan ($p < 0.05$). The recovery session eliminated the sleep deprivation delay ($p < 0.05$).

MSIT performance slowed significantly during acute sleep loss conditions and readily reversed with recovery sleep, indicating that sleep deprivation does have acute yet transient effects on executive function. These results support the notion that total sleep deprivation affects both sustained attention and response inhibition. The improvement from baseline reaction times after sleep recovery may indicate a practice effect not previously described using the MSIT. These readily reversible effects should be explored further in comparison to pathological conditions characterized by irreversible executive functioning deficits, such as stroke and traumatic brain injury.

Disclosures: M. Aulakh: None. **S.R. Fletcher:** None. **C. Eierud:** None. **J. Lisinki:** None. **B. Hamilton:** None. **S.M. LaConte:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.12/JJJ11

Topic: F.01. Human Cognition and Behavior

Support: BMBF Grant 01GQ0411

BMBF Grant 1GQ1001C

DFG Grant GRK1589/1

Title: Neural representation of rules at different hierarchical levels

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Abstract: In everyday life, humans use rules to organize their thoughts and actions in order to achieve specific goals (Bunge & Wallis, 2007). For simple situations, single rules can be used to link a sensory stimulus (the traffic light is green) to its appropriate response (cross the road). More complex situations, however, require the application of multiple rules organized in hierarchies, where high level rules influence the selection or application of lower level rules. Previous studies have demonstrated that Prefrontal Cortex (PFC) is one of the key areas underlying rule processing and control of action. However, it is still unclear whether distinct brain regions within PFC systematically encode qualitatively different task features.
In the present study we investigated whether different features defining a complex rule set are represented in different brain areas depending on the level of control they enforce. To this purpose, we devised an experiment in which participants (N = 20) learnt complex rule sets composed by rules at two different levels of control: low (e.g., "if you see a banana, then press left") and high (e.g., "If you see a star, then only consider red targets"). The task required participants to retrieve, maintain, and apply two rule sets (one low and one high level) to target stimuli. At the beginning of each trial two cues associated with low (or high) level rules were displayed, followed by a delay (delay 1). Then a second pair of cues standing for high (or low) level rules were presented followed by a second delay (delay 2), after which the target was shown. Participants had to apply all the rules to the target stimuli and respond accordingly. The paradigm allowed us to: (i) independently assess the encoding of high and low level rules, (ii) evaluate the difference between the encoding of the two types of rules (comparing high vs. low level rule representations during delay 1, when only one type of rule was maintained), and (iii) decode rule integration (by comparing rule representations during delay 1 vs. delay 2, in which the two levels of rules had to be integrated in order to respond).
We applied multivariate decoding analysis (e.g., Haynes et al., 2007) to functional magnetic resonance imaging data to perform the above-described comparisons. Behavioral as well as

preliminary decoding results suggest that rules at different levels of abstraction are indeed processed differently in distinct brain regions within a large-scale brain network comprising parietal and prefrontal areas.

Disclosures: D. Pischedda: None. **K. Görgen:** None. **J. Haynes:** None. **C.F. Reverberi:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.13/JJJ12

Topic: F.01. Human Cognition and Behavior

Support: R01 NS076665

Title: The brain's response to cognitive demand under drug-induced Impairment: A topiramate study

Authors: *S. E. MARINO[1], C. WANG[2], S.-M. HAN[2], I. SAMUEL[2], J. CIBULA[2], M. DING[2]; [1]Ctr. for Clin. & Cognitive Neuropharm., Univ. of Minnesota, Minneapolis, MN; [2]Univ. of Florida, Gainesville, FL

Abstract: Many CNS drugs negatively impact cognition. How the brain copes with drug-induced impairments is not clear. Understanding this problem is important to explain inter-individual variability in patients' response to these drugs and to optimize therapeutic strategies. We examined this problem with respect to the antiepileptic drug topiramate (TPM) which has also been indicated in a number of other conditions. Healthy volunteers were administered a single, 100-mg oral dose of topiramate in a double-blind, randomized, placebo-controlled, crossover design. High-density scalp EEG was recorded while the volunteers performed a modified Sternberg working memory task with three levels of memory load (1, 3, and 5). Memory retrieval-related ERPs were analyzed across the whole brain. During baseline and placebo conditions, the central-frontal region showed strong memory load modulation; no memory load modulation was observed in bilateral frontal regions. In contrast, during the TPM condition, the memory load modulation in the central-frontal region was significantly reduced but additional brain regions showed sensitivity to memory load, including the bilateral frontal regions. By comparing the ERPs between baseline, placebo and TPM conditions for a given memory load, we further found that during the TPM condition, the ERPs from the central-frontal region were enhanced for low memory load but not for high memory load, whereas the ERPs from the bilateral frontal regions were enhanced for high memory load but not for low memory load. These findings suggest that the brain employed a compensation mechanism by recruiting

more neural resources to cope with the TPM-induced impairment under increasing cognitive demand. For the easy task (memory load=1), the compensation happened within the original task-relevant brain regions (central-frontal). For the difficult task (memory load=5), the neural resources within the original task-relevant brain regions were exhausted due to drug-induced impairment, and additional brain regions were recruited to compensate. Our correlation analysis, which identified a significant correlation across subjects between the compensatory ERP enhancement and the TPM-induced decline in behavioral performance, suggests that the level of compensation under cognitive demand reflects the level of cognitive impairment induced by drug individually.

Disclosures: S.E. Marino: None. **C. Wang:** None. **S. Han:** None. **I. Samuel:** None. **J. Cibula:** None. **M. Ding:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.14/JJJ13

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH082957

Title: Testing the biased competition model of attention in the selection of abstract task rules

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Abstract: Biased competition model was originally proposed as a theory for attentional selection of perceptual information, positing that stimuli within a cell's receptive field compete for cortical representation via mutually inhibitory interactions, and selection results from salience and top-down signals biasing that competition. Although a few studies have shown these principles can also apply to selection of task-relevant perceptual information maintained in working memory (WM), it has not yet been determined whether such mechanisms can also apply to the selection of more abstract types of information in WM. Here we used functional magnetic resonance imaging to investigate whether the selection of abstract, non-sensory information, such as selecting relevant over irrelevant task rules, would result in similar neural patterns predicted by the biased competition model. Participants switched between two different task rules instructed by either the shape or the color dimension of the cues, subsequent to extensive learning of cue-rule mappings. Competition was manipulated by having the color and shape dimensions of the

cue associated either with the same task rule (congruent trials) or different ones (incongruent trials). Phonological and semantic tasks were used as they preferentially activate distinct brain areas, allowing us to measure the magnitude of competition between rules. By comparing incongruent to congruent trials, increased activity was found in a network of brain regions involved in cognitive control, including inferior frontal gyrus (IFG) and parietal cortex. Greater activity in IFG during incongruent relative to congruent trials was associated with a smaller activity difference between incongruent and congruent trials in areas selective for either phonological or semantic task. This finding indicates IFG sends modulatory signals to bias neural activity in areas relevant for the current task rule. When there is competition between task rules, as in the case for incongruent cues, increased activity in IFG is needed to enhance the relevant rule information to what it is when there is no competition for task rule representation, as is the case for congruent cues. In addition, greater activity in IFG for incongruent versus congruent trials predicted the difference in reaction time between incongruent and congruent trials. Overall, these results suggest that "biased competition" may serve as a mechanism to guide the selection of abstract task rule information, biasing the competing rule representations toward the task-relevant over the task-irrelevant ones.

Disclosures: Y. Sheu: None. **S. Courtney:** None.

Poster

573. Working Memory and Executive Function III

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.15/JJJ14

Topic: F.01. Human Cognition and Behavior

Support: R01 MH068721

Title: Increased human hippocampal theta oscillations are associated with the maintenance of temporal order information in working memory

Authors: *L.-T. HSIEH[1,2], A. D. EKSTROM[2,1], K. SHAHLAIE[3], S. T. FARIAS[4], M. SEYAL[4], C. RANGANATH[1,2];
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Abstract: Several electroencephalography (EEG) studies have suggested that neural oscillations might play a role in working memory (WM) processes. In particular, scalp EEG studies suggest that oscillatory activity in the theta band (4-8 Hz) increases during tasks that require the active maintenance of temporal order information, relative to equally difficult tasks that require maintenance of item features. Little is known about the brain regions that are critical for scalp-

recorded theta oscillations, although there is reason to believe that regions in the hippocampus or prefrontal cortex might contribute. Here, we used invasive intracranial EEG recordings from epilepsy patients undergoing presurgical evaluation in order to identify brain regions where oscillatory activity was related to maintenance of temporal order information in WM. Intracranial EEG was recorded while patients completed two types of WM trials: ITEM trials and ORDER trials. On each trial, an instruction word (either "ITEM" or "ORDER") was shown, followed by four sequentially presented visual objects, and then after a 4 sec retention interval, a test display was shown. On ORDER trials, the test display consisted of two visual objects from the previous sequence, and patients were asked to identify which visual object came earlier in the sequence. On ITEM trials, the test display consisted of one previously presented visual object along with another foil object that was not in the sequence. Patients had to identify which of the two objects was presented in the sequence. Preliminary results from three patients show that hippocampal theta was modulated by maintenance of item and order information. Specifically, two out of the three patients showed that the successful maintenance of temporal order information was associated with enhanced hippocampal theta oscillations as compared to the successful maintenance of item information in WM. These results provide preliminary evidence for the importance of hippocampal theta in maintaining the order of recent events. Further analyses will be performed in order to characterize activity in other regions, including the prefrontal cortex.

Disclosures: L. Hsieh: None. **A.D. Ekstrom:** None. **K. Shahlaie:** None. **S.T. Farias:** None. **M. Seyal:** None. **C. Ranganath:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.16/JJJ15

Topic: F.01. Human Cognition and Behavior

Title: Short-term retention of visual information is supported by mechanisms of feature-based attention

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Abstract: Retention of features in visual short-term memory (VSTM) involves maintenance of sensory traces in early visual cortex. However, the mechanism through which this is accomplished is not known. Here, we formulate specific hypotheses derived from studies on feature-based attention to test the prediction that visual cortex is recruited by attentional mechanisms during VSTM of low-level features.
Feature-based attention is characterized by the selective enhancement of responses from sensory neurons preferring an attended feature. Another central characteristic of feature-based attention is the relative suppression of irrelevant (i.e., non-attended) information along the same feature spectrum. If sustained feature-based attention is the mechanism underlying VSTM maintenance of non-spatial information, we should thus observe decreased activity in the feature spectrum surrounding a maintained feature. Here, we tested this prediction using fMRI of early visual cortex. We instructed participants to remember the spatial frequency (SF) of a sample grating to perform a memory discrimination task after a delay interval. During the retention period, a distractor stimulus was presented, and its SF could be identical to the sample, or varied with respect to the sample frequency.
Our results reveal that the blood-oxygen level-dependent (BOLD) responses produced by distractors with SFs in the feature spectrum surrounding the maintained SF are reduced, indicating that neural populations coding for task-irrelevant feature information are suppressed during visual feature memory. The narrow spectral extent of this suppression agrees well with known effects of feature-based attention. Additionally, analyses of effective connectivity during maintenance show that the observed feature highlighting in visual cortex originates in V4, a visual area strongly connected with, and directly modulated by, higher-level attentional control areas such as the frontal eye fields. Furthermore, we show that attentional modulations of earlier visual areas (V1-V3) during maintenance have behavioral consequences, and that these modulations are a result of influences from V4. Again, this corresponds well with the known directions of influence in early visual cortex during attention.
In sum, the present findings fit well with observations from the attention literature, and provide compelling evidence that feature-based attention is the mechanism supporting VSTM maintenance for low-level features in visual cortex.

Disclosures: M.H. Sneve: None. **K.K. Sreenivasan:** None. **S. Magnussen:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.17/JJJ16

Topic: F.01. Human Cognition and Behavior

Support: Friends of BrainHealth 2011 Distinguished New Scientist Award

Title: Causal network dynamics of fluid reasoning components

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Abstract: Reasoning as a multistage cognitive process is involved in identifying rules, patterns, and associations to provide predictable representations of the environment. Several intelligence tests, such as Raven's Progressive Matrices (RPM), indicate considerable variability in reasoning performance among individuals. In characterizing sources of individual differences in reasoning performance, the capacity to process larger sets of problem features or rules has been emphasized. Less attention has been paid to how basic abilities such as change detection (CD), rule verification (RV), and rule generation (RG) affect reasoning performance and the underlying patterns of brain connectivity. We tested this hypothesis using three experimental conditions that additively recruited CD, RV, and RG processing components. Problems consisted of three panels, each containing four geometrical shapes. In CD, participants searched for a shape-change across the panels. In RV, participants verified whether a shape-change followed one of four learned rules, with minimized RG demand. In RG condition, participants inferred whether a shape-change followed a to-be-discovered rule. Hierarchical multiple linear regression analysis of performance measures (accuracy and response time) supported the position that CD, RV, and RG abilities each uniquely account for a portion of variance in RPM performance. Furthermore, functional regions of interest (ROIs) were identified by contrasting the parameter estimates for RV vs. CD, and RG vs. RV conditions. To examine modulation of causal networks in response to the added processing components, multivariate Granger causality analysis was applied to the time series data extracted from the identified ROIs, for each of CD, RV, and RG conditions. Results revealed similar connectivity patterns across task conditions, wherein ROIs such as left-Medial Frontal, left-Inferior Frontal and left-Middle Frontal gyri, as well as bilateral Posterior Cingulate, had central involvement in the causal connectivity networks. Consistent with the additive structure of the task, the number or strength of significant connections increased between CD, RV, and RG conditions, respectively. When the connectivity networks were contrasted between RV and CD conditions, predominately bottom-up connections were identified from posterior brain areas to the frontal cortex. The connectivity contrast between RG and RV revealed top-down influences from frontal ROIs on Middle Temporal and Posterior Cingulate cortices. Finally, our findings provide new insights into the nature of network dynamics essential to fluid reasoning performance.

Disclosures: E. Shokri-Kojori: None. **C. McAdams:** None. **D. Krawczyk:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.18/JJJ17

Topic: F.01. Human Cognition and Behavior

Support: F32MH087047

R01MH081909

Title: Quantitative anatomical evidence for separable dorsolateral and ventrolateral prefrontal networks

Authors: *R. S. BLUMENFELD, M. D'ESPOSITO;
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Abstract: Cognitive Neuroscientific theories drawing on results from the macaque neuroanatomical literature, posit a functional segregation along the dorsal-ventral axis of prefrontal cortex (PFC). However, human functional neuroimaging studies have not provided strong evidence for this segregation, and it is unclear whether the dorsolateral and ventrolateral sub-regions should be considered as part of separate PFC sub-networks. Here we used graph-theoretical community detection analyses on a macaque neuroanatomical dataset to quantitatively test this possibility. These analyses search for partitions of a graph that maximize within community connections and minimize between community connections. The graph we used (Modha and Singh, 2011) was synthesized from the vast amount of macaque neuroanatomical data stored on the CoCoMac database. Examining the graph of connections between frontal nodes, our community detection analyses uncovered a module of lateral PFC nodes. We then submitted this module to the same community detection procedure to look for lateral PFC sub-networks. This analysis revealed distinct partitions that roughly corresponded to dorsolateral and ventrolateral prefrontal with a few exceptions. In addition, a third partition was found that was composed of frontopolar and rostromedial nodes. Subsequently we examined the pattern of connections that the dorsolateral and ventrolateral modules maintain with posterior cortex. Qualitatively these patterns were quite different with very little overlap. Taken together, these results provide evidence that dorsolateral and ventrolateral PFC subregions are separable subnetworks, distinguished both in terms of intra-frontal cortical connections and fronto-posterior cortical connections.

Disclosures: R.S. Blumenfeld: None. **M. D'Esposito:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.19/JJJ18

Topic: F.01. Human Cognition and Behavior

Support: BMBF grant 01GQ0411

SFB 940/1

DFG grant GSC86/1-2009

Title: Human prefrontal cortex independently encodes future task-sequences and their order

Authors: *I. MOMENNEJAD[1], C. REVERBERI[2], J.-D. HAYNES[3];
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Abstract: Every day we need to plan and coordinate multiple future tasks. Previous studies have shown that rostral prefrontal cortex encodes individual future tasks across delays. However, so far it remains unclear how the brain encodes an ordered sequence of upcoming tasks across delays. Here, participants were scanned as they remembered to execute a single future task (single-task trials) or sequences of two tasks (sequence trials). A multivariate classifier was trained on patterns of BOLD activity that encode the single tasks, and was used to decode the identity of the same tasks when they were either first or second in a remembered sequence. The sequential order of tasks (e.g. AB vs. BA) was separately decoded. We found the following. (1) Both the first and the second upcoming tasks could be decoded from rostrolateral prefrontal and parietal cortices. (2) Sequence order was encoded in dorsomedial and dorsolateral PFC. (3) ROI analysis revealed that regions encoding the future tasks and regions encoding sequence order did not overlap. Our findings suggest that (a) the brain encodes future task sequences in a compositional fashion, and (b) there may be a segregation of task and order information in the PFC. Namely, while rostrolateral PFC encoded the identity of multiple future tasks, dorsomedial and dorsolateral PFC encoded information required for the orderly execution of abstract tasks.

Disclosures: I. Momennejad: None. **J. Haynes:** None. **C. Reverberi:** None.

Poster

573. Working Memory and Executive Function III

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.20/JJJ19

Topic: F.01. Human Cognition and Behavior

Support: DOD ICB Contract W911NF-09-D-0001

Title: The neural basis of hypothesis formation and evaluation

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Abstract: Research on split-brain, delusional, and stroke patients suggests that the left hemisphere may concoct stories, or hypotheses, to explain contradictory or ambiguous events, while the right hemisphere may evaluate the likelihood and validity of the left hemisphere's hypotheses. The goal of the present study was to localize the neural substrates of hypothesis formation and evaluation in healthy participicants. Based on studies of inference making and deductive reasoning, we predicted that the left dorsal medial prefrontal cortex (dmPFC) supports hypothesis generation, and that either the right dmPFC or right inferior PFC supports hypothesis evaluation, as these areas are associated with conclusion validation and belief-logic contradiction. In this study, we recorded subjects' brain activity with fMRI as they attempted to generate appropriate category labels for a series of word sets. We used novel word sets that were designed to either elicit hypothesis formation and evaluation ("ad hoc" word sets) or automatic processing ("automatic" word sets). We found that the ad hoc word sets were associated with longer response times - that is, subjects needed more word evidence to generate appropriate category labels - and lower accuracy ratings than the automatic word sets. Based on our assumption that subjects create and verify hypotheses to a greater extent 1) during the period before generating a possible label and 2) during ad hoc trials, we looked for differential brain activity in ad hoc trials vs. automatic trials and TRs before vs. after label generation. Consistent with our predictions, both contrasts revealed increased activity in the left dmPFC, right dmPFC, and left vlPFC, which we attribute to hypothesis formation, hypothesis evaluation, and memory retrieval, respectively. The results of this study provide a focally-specific neural explanation for the behaviors associated with split-brain and delusional patients.
This research was supported by the Institute for Collaborative Biotechnologies through contract W911NF-09-D-0001 from the U.S. Army Research Office.

Disclosures: N. Marinsek: None. **B.O. Turner:** None. **M.B. Miller:** None.

Poster

573. Working Memory and Executive Function III

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.21/JJJ20

Topic: F.01. Human Cognition and Behavior

Support: IARPA D10-PC20021

Title: Attentional saliency modulates non-Bayesian updating and sequencing behaviors in a large-scale neurocognitive model

Authors: *M. PHILLIPS[1], R. UHLENBROCK[1], M. ZIEGLER[1], Y. SUN[2], H. WANG[2], R. THOMSON[3], C. LEBIERE[3], R. BHATTACHARYYA[1];
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Abstract: Several discrete and complementary behaviors are thought to arise when humans sequence, process and update their beliefs across a set of competing hypotheses. For instance, it has been suggested that preparation and the amount of processing required determine the processing order of stimuli (Leonhard 2011; Fortin and Masse 1999). Attentional modulation has also been shown to play a central role in the processing order of stimuli in a verbal short-term memory task (Majerus et al. 2006). However, a comprehensive and neurobiologically-detailed description of these behaviors has thus far been lacking. Here, individual and phenotypic differences in updating strategy, sequencing, and processing order were investigated in a variant of the n-arm bandit task. Human subjects typically adopted one of two updating strategies for probability estimation (normalized reporting - probabilities sum to 100%, and non-normalized reporting). Non-normalizers utilized a non-Bayesian, attentional-based saliency measure in processing order for sequential hypothesis updating. To explore the biological basis of these behaviors, a neural model which sequences, processes, and updates the beliefs of competing hypotheses was created within a large-scale neurocognitive model. Saliency-based ordering and attentional sequencing was modeled in the inferior parietal and medial frontal cortical regions (including the supplementary motor area) consistent with current theories of non-motor sequencing (Tracy et al. 2011). The model reliably reproduced the discrete human behavioral phenotypes observed in sequential hypothesis updating. These results highlight the importance of attentional saliency in sequencing and updating behaviors and provide a simulation framework for future studies to investigate attentional saliency in other high-level cognitive behaviors.

Disclosures: M. Phillips: None. **R. Uhlenbrock:** None. **M. Ziegler:** None. **Y. Sun:** None. **H. Wang:** None. **R. Thomson:** None. **C. Lebiere:** None. **R. Bhattacharyya:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.22/JJJ21

Topic: F.01. Human Cognition and Behavior

Title: Effect of sound pressure level on brain function during memory task using fNIRS

Authors: *F. INOUE, U. YAMAMOTO, T. HIROYASU;
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Abstract: [Purpose]
In this study, brain functions were examined during a numerical memory task within a sound environment, including the effect of sound pressure level.
[Experimental Methods]
In this study, a numerical memory task was chosen as the working task. The task shows eight random digits that subjects have to memorize. Subjects performed the task in two types of sound environments, namely, white noise and "sonata for two pianos in D major (Mozart)" composed by Mozart. Task scores and oxyhemoglobin concentration
caused by changes in cerebral blood flow were analyzed in both sound environments. The sound environment levels were 65 dB (white noise) and 75 dB (Mozart). Cerebral blood flow changes were measured using functional near infrared spectroscopy.
[Results]
There were significant differences between the two types of sound pressure levels in terms of task score and cerebral blood flow change. The difference was shown when Mozart was presented and the difference was not shown when white noise was presented. In addition, an increase in the oxyhemoglobin concentration was found in both the sound environments.
Therefore, the sound types can be classified into two classes, namely, sounds that do not easily affect subjects and sounds that do. In conclusion, while studying task performance and cerebral blood, the sound environment must be carefully chosen.

Disclosures: F. Inoue: None. **U. Yamamoto:** None. **T. Hiroyasu:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.23/JJJ22

Topic: F.01. Human Cognition and Behavior

Title: Longer reaction time is associated with increased task-specific cognitive control and decreased default mode activity

Authors: *A. D. BARBER[1,2], B. S. CAFFO[3], J. J. PEKAR[1,2], S. H. MOSTOFSKY[1,2];
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Abstract: Within-subject, trial-to-trial fluctuations in reaction time (RT) may reflect fluctuations in attention, integration of information across regions that implement different aspects of the task, or state changes in global network efficiency. Previous studies have found that specific regions are influenced by RT independent of task demands, which may reflect the particular function of the region (e.g. response selection demand). The current study examined RT effects on brain activity in two Go/No-go tasks: a Simple task with an intuitive stimulus-response mapping (green=go, red=no-go) and a Repeat task, with an inconsistent stimulus-response mapping (color change=go, color repeat=no-go), which required working memory for task performance.

22 healthy adults were scanned for two blocks on each task. Image preprocessing and analysis occurred in SPM5. First-level general linear models included up to seven condition trial onset regressors (Post-Rest Go, Go, Go RT, No-go, Commission Error, Omission Error, and Anticipatory trials on which the RT<200 msec), which were convolved with the canonical HRF, temporal and dispersion derivatives. In addition, nuisance regressors (six motion parameters, mean white matter, mean cerebrospinal fluid, and mean whole brain time-courses) and a block regressor for each functional run were included. First-level contrasts of Go RT revealed those regions that increased or decreased activity linearly with RT for the Simple and Repeat Tasks separately. Second-level group effects were examined across subjects.

In the Simple Task, slower RT was associated with increased activation in visual (BA 19), inferior and superior temporal (BA 37/39/22), inferior and superior parietal (BA 7/40), and postcentral gyrus (BA 5/2). In the Repeat Task, activation increases with RT occurred within a right frontal region spanning dorso- and ventrolateral cortices (BA 9/44/45/46), anterior insula (BA 13), pre-supplementary motor area and anterior cingulate (BA 6/8/32) and bilateral parietal regions mainly confined to inferior and superior parietal cortex (BA 7/40) with greater extent into supramarginal and angular gyri on the right side. Examination of regions that showed decreased activity with slower RT revealed the medial prefrontal cortex (MPFC: BA 9/10/32) for both tasks.

RT modulated activity in a unique set of regions for the two tasks, reflecting task-specific cognitive/attention control. Increased recruitment of these regions may reflect greater deliberate control in slow RT trials. Decreased MPFC activation in slow RT trials may reflect default mode network suppression when control increased.

Disclosures: A.D. Barber: None. **B.S. Caffo:** None. **J.J. Pekar:** A. Employment/Salary (full or part-time):; Philips. **S.H. Mostofsky:** None.

Poster

573. Working Memory and Executive Function III

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.24/JJJ23

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH096801

Title: Multi-task functional connectivity reveals the human brain's dynamic network architecture and stable functional backbone

Authors: *M. W. COLE[1], D. S. BASSETT[2], J. D. POWER[1], S. E. PETERSEN[1];
[1]Washington Univ., ST LOUIS, MO; [2]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Functional connectivity - the statistical dependence among brain activity time series - has been investigated extensively during task performance. However, task-state functional connectivity has not revealed general network architectural properties of human brain function outside of specific task contexts. This is in contrast to network properties identified in the resting-state, which researchers assume will generalize across many contexts, making them broadly functionally relevant. To identify general network architectural properties during task performance, we measured brain activity during a task paradigm involving 64 distinct task states with functional MRI. Using community detection algorithms developed for such multiplex network scenarios, we found that brain regions were assigned to different putative functional modules across task states, highlighting the human brain's flexibility in adaptively altering functional connectivity patterns to meet external task demands. Moreover, these assignments differed significantly from those identified in resting-state functional connectivity, suggesting that the human brain's dynamic repertoire cannot be fully characterized using static resting-state functional connectivity estimates. To identify consistent patterns of functional connectivity across task states, we utilized a tuning function in the multiplex community detection algorithm and found that consensus assignments showed statistically significant similarity to the resting-state assignments as measured by the z-score of partition similarity. These results suggest that while resting-state functional connectivity estimates cannot fully account for the human brain's dynamic network architecture, it nonetheless provides important information regarding the central tendency of that architecture across many task states. Resting-state functional connectivity might therefore reflect a stable functional backbone from which the wide range of task-related reconfigurations stem.

Disclosures: M.W. Cole: None. **D.S. Bassett:** None. **J.D. Power:** None. **S.E. Petersen:** None.

Poster

573. Working Memory and Executive Function III

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.25/JJJ24

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant NS40813 to MD

NDSEG to CG

Title: Focal lesions lead to functional plasticity in the roles of individual brain regions within large-scale networks

Authors: *C. GRATTON, M. D'ESPOSITO;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Focal lesions can change functional brain interactions, even in sites remote from the structural damage. Graph theoretical tools can be used to quantify network reorganization after damage. Global metrics such as Newman's modularity measure the degree to which the brain can be decomposed into separable large-scale networks. Nodal metrics, such as participation coefficient (PC) and within module degree (WD), examine the roles of individual regions play within this structure. Regions with many between-module connections (measured by PC) are connectors and regions with many within-module connections (measured by WD) are hubs.
In past work, we demonstrated that lesions impact the brain's network structure, decreasing modularity in patients compared with healthy controls and in the lesioned compared with the non-lesioned hemisphere. Furthermore, these effects are related to the properties of the damaged region, with stronger decreases seen with more connector damage. Here, we extend these findings by testing if these global changes are accompanied by local changes in the roles of individual regions. Functional plasticity in lesion patients may occur at multiple levels: in regions proximal to, homologous to, or within the same network as the sites of structural damage. We recorded a period of resting state fMRI data from a group of patients with focal lesions (N = 35) and healthy controls (N = 24). Time-series correlations were measured between each of ~90 anatomical regions. This served as the basis for creating unweighted undirected graphs. Graphs were partitioned into their optimal modules. Within this structure, we quantified the PC and WD properties of each brain region.
Results showed that widespread changes were present in the PC and WD properties of the lesioned brain. Changes were systemically related to the healthy roles of each region: the most connector and hub like regions were those most likely to decrease connector and hub properties

in patients. Furthermore, the change in the roles of regions differed between hemispheres. On average, the non-lesioned, compared with the lesioned, hemisphere shifted towards regions having more connector and less hub like properties. These changes were particularly strong in the nodes homologous to the lesion, which significantly increased in both their connector and hub properties.
These results suggest that global changes in the large-scale network organization of the brain are accompanied by local changes in the roles that regions play within these modules. This provides support for the idea that focal brain damage can cause systematic functional plasticity in the roles of non-damaged locations.

Disclosures: C. Gratton: None. **M. D'Esposito:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.26/JJJ25

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01MH085328

Title: Global brain organization is disrupted in children with ADHD

Authors: *J. R. COHEN[1], A. D. BARBER[2], M. B. NEBEL[2], M. D'ESPOSITO[1], S. H. MOSTOFSKY[2];
[1]Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA; [2]Kennedy Krieger Inst., Baltimore, MD

Abstract: Attention deficit hyperactivity disorder (ADHD) is the most commonly diagnosed developmental disorder of childhood, affecting approximately 5% of children worldwide. ADHD is hypothesized to result from dysfunctional connectivity patterns within and between brain networks. Most existing research focuses on specific networks or pairs of brain regions, despite strong evidence that ADHD is associated with a distributed pattern of abnormality across much of the brain. Therefore, the goal of this study was to compare global measures of network organization across the entire brain in children with ADHD and typically developing (TD) children to determine how dysfunctional brain organization in an intrinsic (resting) state, unrelated to specific cognitive processes, may be associated with ADHD.
We collected five minutes of resting state data in children with ADHD and TD children undergoing fMRI. For analyses, groups were matched on the basis of number of participants, age, gender, and average motion during the scan. We applied graph theoretical tools to calculate

global measures of network organization across the entire brain. We focused on three measures: modularity, clustering, and global efficiency. Modularity is a measure of the brain's organization into separate, densely-connected networks (or modules) with only sparse connections across networks. Clustering is a measure of how inter-connected neighboring nodes are. Global efficiency is a measure of how few steps it takes to link any pair of nodes together (higher global efficiency indicates more long-range connections).
Modularity was significantly increased in children with ADHD as compared to TD children. When separately examining within-network connections (clustering) and long-range connections (global efficiency), children with ADHD displayed increased clustering and decreased global efficiency as compared to TD children. Taken together, these findings indicate that children with ADHD, as compared to TD children, show increased within-network connectivity and decreased connectivity across networks.
These findings imply that children with ADHD have decreased integration across distinct intrinsic networks, even in the absence of specific cognitive demands. Such integration is critical for complex cognitive processes, such as response control and working memory, processes that are impaired in ADHD.

Disclosures: J.R. Cohen: None. **A.D. Barber:** None. **M.B. Nebel:** None. **M. D'Esposito:** None. **S.H. Mostofsky:** None.

Poster

573. **Working Memory and Executive Function III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 573.27/JJJ26

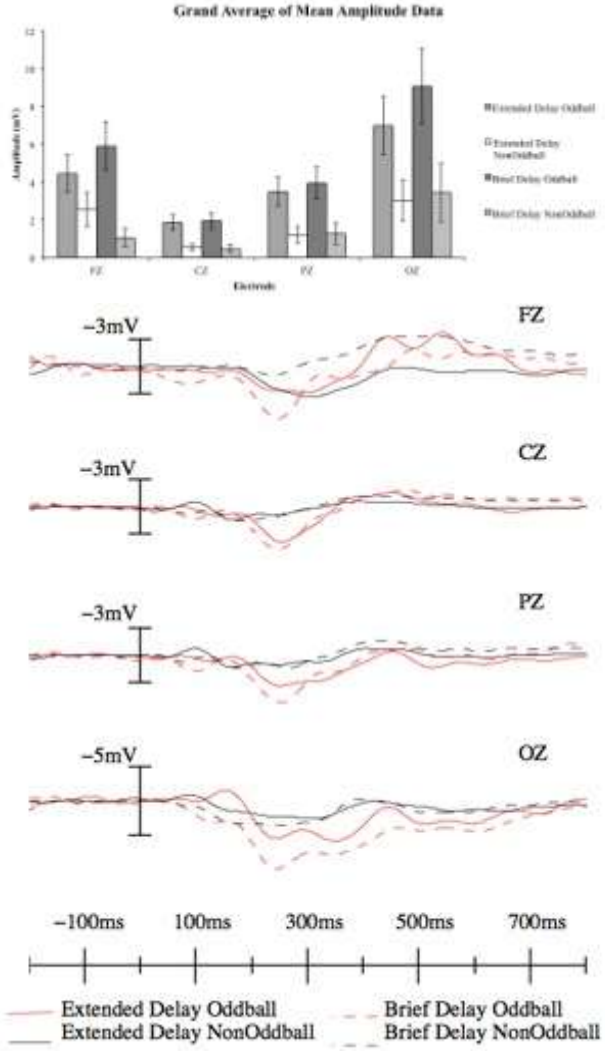
Topic: F.01. Human Cognition and Behavior

Title: Assessing serial order processing using a novel P3 oddball paradigm: A proof of concept investigation

Authors: *W. C. HOCHBERGER[1], J. AXELROD[2], T. CARRATHERS[2], S. HILL[2];
[1]Dept. of Clin. Psychology, [2]Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Serial order recall is a critical component for establishing temporal order of stimuli and interacting appropriately and effectively in the environment. Recent findings have demonstrated that deficits in serial order processing go beyond the pervasive general cognitive deficits seen in schizophrenia. A traditional oddball task was implemented to assess a key component of serial order processing. The novelty of this task lies in the utilization of electroencephalography (EEG) as an indicator of maintenance of working memory serial order

information and potentially for differential degradation of working memory stores during specific processing conditions. As hypothesized, the amplitude of the P3 was greater in oddball trials compared to nonoddball trials across both processing conditions. In addition there was an interaction effect in which the amplitude of the P3 was larger when visual presentation was not externally paced, suggesting that externally pacing target recognition increases cognitive demand and results in a more rapid "exhaustion" of the P3 system. Taken together these findings suggest that the novel task is both an effective means of eliciting the P3 and represent a new way of assessing serial order processing at a physiological level.



Disclosures: W.C. Hochberger: None. **J. Axelrod:** None. **T. Carrathers:** None. **S. Hill:** None.

574**Poster**

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.01/JJJ27

Topic: F.02. Animal Cognition and Behavior

Support: Grant-in-Aids for Scientific Research (KAKENHI, No. 25240021) to S.F

Grant-in-Aids for Scientific Research (KAKENHI, No. 21240024) to S.F

JSPS Research Fellowships for Young Scientists to K.W.

Title: Primate prefrontal activity during simultaneous performance of spatial attention and spatial working memory tasks

Authors: *K. WATANABE[1,2,3], S. FUNAHASHI[3];
[1]The Japan Society for the Promotion of Sci. (JSPS), Tokyo, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Kokoro Res. Ctr., Kyoto Univ., Kyoto, Japan

Abstract: Despite its remarkable flexibility, human cognitive information processing is severely capacity-limited. When conflicting and interfering streams of information have to be processed concurrently in dual-tasks, there are evident behavioral signs of capacity overload, such as a decrease in percent correct rates and prolonged response times relative to those in individual tasks performed separately. Since this effect, known as dual-task interference, is thought to be a direct proof of cognitive capacity limitation, dual-task performance has been extensively investigated in cognitive psychology and functional neuroimaging studies. Although earlier studies consistently proposed that the lateral prefrontal cortex (LPFC) played a key role in dual-task performance and interference, the underlying neuronal mechanisms remain almost completely unknown due to a lack of direct neurophysiological investigations. In this study, we recorded and analyzed single-neuron activities in LPFC while monkeys performed dual-tasks that required the simultaneous performance of a spatial attention task and a spatial memory task. We found that the performance of the monkeys exhibited dual-task interference, and prefrontal neuron activities showed a decreased ability to represent task-relevant content (i.e., spatial location) to a degree proportional to the increased demand of the concurrent counterpart task even in correct trials. The neural locus of the interference was identified to reside in the simultaneous, overloaded recruitment of the same LPFC neural population by the two tasks. These results indicate that information processing capacity of LPFC single-neuron activity is (1) limited to a fixed level, below that fully accommodates information of two concurrent tasks, (2) adaptively allocated between tasks in graded quantity, (3) enhancing behavioral performance as its allocation to one task increases. The present study provides direct neurophysiological evidence for, and constraints to, psychological models of dual-task interference and capacity limitation.

Disclosures: K. Watanabe: None. **S. Funahashi:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.02/JJJ28

Topic: F.02. Animal Cognition and Behavior

Support: DFG grant NI 618/2-1

Title: Exploring numerosity representation in the prefrontal and parietal cortices of numerically naïve monkeys

Authors: *P. VISWANATHAN, A. NIEDER;
Animal Physiol., Univ. of Tübingen, Tübingen, Germany

Abstract: While recent years brought significant advances in our understanding of the neural substrates and mechanisms that represent numerical quantities, little is known about the development of numerosity selectivity in the brain. Work from our group has shown that single neurons in the parietal and frontal association cortices encode visual set sizes. So far, these investigations have been done exclusively in animals able to discriminate magnitude, consequently raising the possibility that aspects of neuronal numerosity selectivity might be shaped by learning and experience. Whether numerosity detectors similar in number and response characteristics also exist in naïve animals remains untested.
Here, we investigate neuronal numerosity selectivity in numerically naïve rhesus monkeys i.e. monkeys that never learned to discriminate numerosity. The monkeys performed a delayed match-to-sample-color task where they matched the color of a sample array to that of test arrays presented sequentially after a delay period. To address spontaneous selectivity to numerical quantity, the stimulus displays consisted of visual arrays of colored dots with varying numbers of items from one to five. The monkeys received no numerosity training and no relevance was associated with the numerosity information in the stimuli. We recorded multi-channel single-unit activity from the dorsolateral prefrontal cortex (PFC) and the ventral intraparietal area (VIP) of the posterior parietal cortex while the monkeys performed the color discrimination task. Based on average discharge rates during sample period, a three-factorial analysis of variance (ANOVA, $p < 0.01$) was calculated for every neuron with numerosity (one to five), color (five different colors) and stimulus protocol (standard vs. size and density control) as factors
Neurons recorded from PFC and VIP showed significant modulation of discharges to numerosity. This suggests visual numerosity is a natural perceptual category extracted by an intuitive “visual sense of number”. Numerosity-selective neurons were spontaneously tuned to preferred numerosities. This finding argues for the labeled-line code to be a default mechanism

of numerosity encoding rather than an artifact of discrimination training. Finally, we found that numerosity is encoded earlier in the VIP, suggesting that numerical information is automatically extracted in the parietal cortex, and then conveyed to the frontal lobe.

Disclosures: P. Viswanathan: None. **A. Nieder:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.03/JJJ29

Topic: F.02. Animal Cognition and Behavior

Support: NI 618/2-1

JA 1999/1-1

Title: Dopamine modulates numerical rule coding in the primate prefrontal cortex

Authors: T. OTT, S. N. JACOB, *A. NIEDER;
Univ. Tuebingen, Tuebingen, Germany

Abstract: Numerical competence, the capability to use quantities and numbers, is based on highly abstract principles, or rules, of how to structure, process and evaluate quantitative information. Numerical competence thus inherently requires executive control functions. We have shown previously that single neurons in the primate prefrontal cortex (PFC) encode abstract numerical rules. The cellular mechanisms giving rise to the rule-related neuronal activity are, however, poorly understood. Since the PFC receives strong projections from the dopaminergic midbrain modulating executive functions such as working memory, we hypothesized that dopamine receptors in the PFC are involved in regulating abstract rule coding.
We trained two rhesus monkeys (Macaca mulatta) to compare numerosities and to switch flexibly between two abstract numerical rules based on a rule cue. The “greater than” rule required the monkeys to release a lever if the first test display showed more dots than the sample display, whereas the “less than” rule required a lever release if the number of items in the test display was smaller compared to the first test display. We recorded single neurons in the lateral PFC while simultaneously applying the dopamine D1 receptor (D1R) agonist SKF81297 or the D1R antagonist SCH23390 to the vicinity of the cells using microiontophoresis.
Our data show that after application of the D1R agonist, neuronal activity related to the neurons’ preferred numerical rules was enhanced. Importantly, the quality of rule coding was significantly improved by stimulating the D1R compared to control conditions. Furthermore, recordings using

the D1R antagonist showed that blocking D1Rs in the PFC impairs numerical rule coding by reducing neuronal activity to the neurons' preferred rules. Together, these results show that abstract rule coding in the PFC relies on D1R stimulation. Thus, dopamine might be involved in modulating the neuronal underpinnings of executive functioning in the PFC to guide behaviour.

Disclosures: T. Ott: None. **A. Nieder:** None. **S.N. Jacob:** None.

Poster

574. Executive Function: Corticostriatal Mechanisms

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.04/JJJ30

Topic: F.02. Animal Cognition and Behavior

Support: DFG Grant JA1999/1-1

Title: Protected storage of working memory in the primate parietal, not prefrontal cortex

Authors: *S. N. JACOB[1,2], A. NIEDER[1];
[1]Tuebingen Univ., Tuebingen, Germany; [2]Dept. of Psychiatry, Charité, Berlin, Germany

Abstract: The primate lateral prefrontal cortex (PFC) and posterior parietal cortex (PPC) are major nodes in a brain network that supports short-term memory. While PFC has been shown to protect important memory content from distracting stimuli, PPC is driven more strongly by sensory inputs, irrespective of their relevance to the current task. We compared extracellular single-unit activity from the PFC and the ventral intraparietal area (VIP) of two rhesus monkeys trained to memorize and discriminate visually presented set sizes (numerosities). While performing the task, the animals had to suppress a salient distractor numerosity that appeared during the memory delay. Surprisingly, we found that target memories encoded in PFC ensemble activity were not maintained throughout the trial, but that distractors replaced target information as soon as they were presented. In contrast, only few VIP neurons represented the distractor stimuli: the majority of VIP neurons carried target, but not distractor information at various time points in the trial. Importantly, accumulation of target memory information in VIP, not PFC, correlated with behavior and predicted whether the animal would successfully complete a trial or commit an error. Our results challenge the commonly held view that resistance to inference in PFC is the key mechanism for selection and maintenance of task-relevant stimuli in working memory. Instead, our findings suggest that parietal association cortex can also function as a protective storage for complex abstract memories and thus guide behavior.

Disclosures: S.N. Jacob: None. **A. Nieder:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.05/JJJ31

Topic: F.02. Animal Cognition and Behavior

Title: The function of the medial prefrontal cortex during a working memory task in sexually motivated male rats

Authors: *M. L. ALMANZA[1], M. HERNANDEZ-GONZALEZ[1], M. E. OLVERA-CORTES[2], B. E. GUTIERREZ-GUZMAN[2], M. A. GUEVARA[1];
[1]Inst. De Neurociencias, Guadalajara, Mexico; [2]Ctr. de Investigacion Biomedica de Michoacan, Michoacan, Mexico

Abstract: The medial prefrontal cortex (mPFC) is involved in organizing delayed responses and, consequently, in working memory function; i.e., the type of memory that allows relevant information to be stored for a short time in order to carry out a certain task that requires immediacy and entails decision-making, but can be erased once the task is completed. Most of the studies that have evaluated working memory in rats have used food or drink as rewards; however, sexual behaviour has also proven to be a highly-rewarding motivated behaviour that has been used efficiently as both an incentive and as a reward in classical operant tasks, such as mazes and Skinner boxes. This work investigated if functional activity of the medial prefrontal cortex (mPFC) changes in relationship to working memory processes involved in the performance of a sexually motivated task in male rats. Male Wistar rats that were bilaterally implanted in the mPFC were submitted to a non-matching sample task in a T-maze using as reinforcer sexual interaction with a receptive female during 4 training days. Based on their performance throughout the training days in the task, the rats were classified in two groups, good-learners and bad-learners. Only the good-learning rats showed an increase in the absolute power of the 8-13 Hz band during the sample-run and test-run, which could be related with the learning of the working memory elements of the task. The good-learner rats showed also, only during the maintenance phase (when the rule was learned), an increased correlation of the 8-13 Hz band during the sample run, indicating that a high coupling between prefrontal cortices is necessary to the adequate processing that allows the rats make the correct decision in the maintainence phase. Together, these data show that mPFC activity changes in relationship to the working memory processes involved in a sexually motivated task of male rats.

Disclosures: M.L. Almanza: None. **M. Hernandez-Gonzalez:** None. **M.E. Olvera-Cortes:** None. **B.E. Gutierrez-Guzman:** None. **M.A. Guevara:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.06/JJJ32

Topic: F.02. Animal Cognition and Behavior

Support: New Ventures, UNH

Title: Memory coding properties of central thalamic neurons: relationship to prefrontal neuronal coding

Authors: R. L. A. MILLER, C. J. BATES, K. D. ONOS, B. A. WORMWOOD, M. J. FRANCOEUR, B. M. GIBSON, *R. G. MAIR;
Univ. New Hampshire, Durham, NH

Abstract: Central thalamus influences prefrontal cortex (PFC) through specific projections of the mediodorsal nucleus (MDn) to middle cortical layers and nonspecific projections of the rostral intralaminar nuclei (ILn) to deep and superficial layers. The MDn is reciprocally connected to widespread areas of PFC and is an important target of pallidal areas driven by prefrontal projections. The ILn are also reciprocally connected to PFC and additionally innervate areas of the basal ganglia that receive projections form PFC. Earlier studies in our lab and other labs have demonstrated that lesions damaging MDn and ILn interfere with aspects of spatial memory that depend on PFC. Here we extend this research by comparing memory-coding properties of MDn and ILn neurons with results for PFC neurons using common behavioral measures and recording procedures. Here we used moveable tetrode arrays to compare the memory-coding properties of neurons in MDn and ILn. Rats were trained to perform a right/left delayed non-matching to position (DNMTP) task in octagonal chambers with retractable levers located on the N, E, S, and W sides. The location of the lever used to initiate each trial was varied at random to distinguish between egocentric and allocentric navigation. Recording arrays consisted of four tetrodes two each in separate cannulae, one aimed more medially at the MD and central medial nuclei and one aimed more laterally at the centrolateral and paracentral nuclei. Recording implants consisted of four tetrodes mounted in an acrylic base attached to machine screws that allowed them to be lowered gradually in approximately 60 steps through thalamus. TTL pulses were generated to mark specific behavioral events within the task in order to correlate activity from identified discrete neurons. Data analyses entailed the use of Klustakwik spike sorting software and examination of raster plots and peri-event time histograms based on behavioral events. Behavioral analyses have also been conducted using time-stamped video recordings made throughout all recording sessions. Our results allow direct comparison

between MDn and ILn neurons with results from a parallel study of memory coding properties of PFC neurons.

Disclosures: R.L.A. Miller: None. **C.J. Bates:** None. **K.D. Onos:** None. **B.A. Wormwood:** None. **M.J. Francoeur:** None. **B.M. Gibson:** None. **R.G. Mair:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.07/JJJ33

Topic: F.02. Animal Cognition and Behavior

Support: New Ventures, UNH

Title: Memory-coding properties of prefrontal cortical neurons in the rat

Authors: *K. D. ONOS, B. A. WORMWOOD, R. L. A. MILLER, M. J. FRANCOEUR, E. F. HEBERT, A. W. BLAKE, B. M. GIBSON, R. G. MAIR;
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Abstract: Prefrontal cortex (PFC) supports a number of aspects of executive function, including working memory. PFC, as defined by the projection areas of the mediodorsal thalamic nucleus (MDn), consists of a diverse series of cortical fields that line the medial and ventral surfaces of frontal cortex in the rat. Here we used moveable tetrode arrays to compare the memory-coding properties of neurons in MDn projection areas along the medial wall of rat PFC. Rats were trained to perform a right/left delayed non-matching to position (DNMTP) task in octagonal chambers with retractable levers located on the N, E, S, and W sides. By randomly varying the location of the lever pressed to start trials, we are able to distinguish between egocentric and allocentric coding. Recording arrays consisted of four tetrodes oriented vertically down the medial wall, 3 mm anterior to Bregma in the left and right hemispheres. The tetrodes were mounted in plastic bases with a tripod of machine screws that allowed them to be lowered gradually in approximately 80 steps through cingulate, prelimbic, and infralimbic areas of PFC. Signals originating from single neurons were identified with Klustakwik spike sorting software and analyzed as raster plots and peri-event time histograms based on TTL pulses marking significant behavioral events. Behavioral analyses have also been conducted using time-stamped video recordings made throughout all recording sessions. Our results have mapped several populations of neurons on to the cortical fields studied. These include: responses occurring just before the start of DNMTP trials, increased activity during periods of movement between

locations of levers, delay period activity related to the direction of turning during correct choice responses, and activity associated with lever pressing and positive reinforcement (water).

Disclosures: K.D. Onos: None. **B.A. Wormwood:** None. **R.L.A. Miller:** None. **M.J. Francoeur:** None. **E.F. Hebert:** None. **A.W. Blake:** None. **B.M. Gibson:** None. **R.G. Mair:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.08/JJJ34

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Inactivation of medial prefrontal cortex or acute stress impairs performance of an odor span task in rats

Authors: D. A. DAVIES[1], J. J. MOLDER[1], Q. GREBA[1], *J. G. HOWLAND[2];
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Abstract: Working memory is a type of short-term memory for storage and manipulation of information necessary for higher order cognition. The capacity of working memory is often assessed by measuring the "span" or number of stimuli that can be retained in working memory. The present experiments examined whether either reversible inactivation of medial prefrontal cortex (mPFC) or acute stress impaired working memory performance using an odor span task (OST) in rats. The OST requires rats to remember an increasing span of different odors to receive food reward. Briefly, a within subjects design was used with two separate batches of male Long Evans rats (experiment one: mPFC inactivation, n=13; experiment two: acute stress, n=7). Rats were food restricted for all experiments. The rats used for the mPFC experiment had cannulae implanted into mPFC using conventional techniques. After shaping the rats to dig in unscented sand for a food reward, the rats were trained in a delayed non-match to sample procedure. The rats were permitted to dig in a scented cup of sand on a platform for a food reward. After retrieving the food, the rat was removed from the platform and placed behind a curtain to block its view of the platform. The experimenter then moved the cup to a random position on the platform and added a second cup with a novel odor. The rat's task was to choose the cup with the novel odor. After rats reliably performed the delayed non-match to sample procedure, subsequent test days involved adding additional cups with novel scents one at a time

until an error was made. The number of cups that the rat correctly chose before it made an error (not counting the first cup) was recorded as the span for that trial. Experiment one used microinfusions (0.50 μl; 30 min before testing) of the GABA agonists muscimol and baclofen to reversibly inactivate the mPFC. In experiment two, 30 min of restraint stress was administered 30 min before testing. Medial PFC inactivation impaired performance of the OST. The mean spans observed following sham, vehicle, or GABA agonist infusions were 8.23 odors, 6.49 odors, and 0.50 odors, respectively. In experiment 2, the unstressed rats displayed a mean span of 7.07 odors while the stressed rats had a mean span of 4.55 odors. The present results demonstrate a critical role for the mPFC in the odor span task. Future studies using the OST would shed light on the molecular mechanisms in mPFC underlying working memory and its disruption by acute stress. Such studies may result in novel therapeutic options for individuals with impaired working memory.

Disclosures: D.A. Davies: None. **J.G. Howland:** None. **J.J. Molder:** None. **Q. Greba:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.09/JJJ35

Topic: F.02. Animal Cognition and Behavior

Support: 5R01MH073689-07

5P50MH094263-02

Title: Prefrontal and striatal interactions during habit learning and strategy switching

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Abstract: Human behavior can be guided by previous experience to form either procedural or episodic memory, based on different types of information learned in distinct conditions, and processed by dissociable brain systems. Patients with medial temporal lobe (MTL) damage cannot learn new facts, but learn and retain motor skills. The opposite pattern is seen in patients with basal ganglia dysfunction, e.g. in Parkinson's or Huntington's disease. Similarly, homologous dissociations exist in other primates and rats, such that the striatum is central to procedural learning whereas the hippocampus governs episodic learning. The neural mechanisms that allow normal subjects to switch seamlessly between these two sources of information are

unknown. Damage to specific regions of the prefrontal cortex impairs different aspects of behavioral flexibility in people and in animals. Damage to the dorsolateral prefrontal cortex in primates or the analogous medial prefrontal cortex (mPFC) in rats does not impair either procedural or episodic learning, but does impair switching between the two strategies. The dorsal striatum supports multiple memory systems beyond the procedural learning system. The cortex and striatum are connected through distinct parallel loops: sensorimotor, limbic, and cognitive (Alexander, Crutcher, & DeLong, 1990; Retailleau, Etienne, Guthrie, & Boraud, 2011). While the dorsolateral striatum (DLS) is required for incremental, procedural memory developed by stimulus-response associations, the dorsomedial striatum is required for spatial memory. Executive tasks such as set-shifting are impaired in early PD, as seen in the Wisconsin Card Sort Task (Grahn, Parkinson, & Owen, 2009; Roca et al., 2012). This leads to the inevitable question—is the PFC dysfunctional in a basal ganglia disorder, or is the striatum necessary for executive function? The role of the DLS in learning to switch between memory strategies is unknown. Using a plus maze, we have shown that DLS lesions, as expected, impair the ability to learn a stimulus-response association. However, the DLS was not required for strategy switches. Future work can use multi-site electrophysiological recordings to understand how the PFC and DLS interact upon use and switching of the procedural or habit strategy.

Disclosures: K.S. Casten: None. **M. Shapiro:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.10/JJJ36

Topic: F.02. Animal Cognition and Behavior

Support: Bernstein Center for Computational Neuroscience Grant #FKZ:01GQ1002

Deutsche Forschungsgemeinschaft Grant #NI618/4-1

Title: Neural activity of abstract quantitative rules applied to spatial and numerical magnitudes: Comparison of prefrontal, premotor and cingulate motor cortex

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Abstract: Processing quantity information based on abstract principles is central to intelligent behavior and a hallmark of executive control. We have previously shown that such quantitative rules are encoded in the lateral prefrontal cortex (PFC). However, the PFC might not process

rule-related information in isolation. Other cortical areas might as well contribute to such higher order strategic behavior. Recent studies suggested that e.g. premotor areas might encode rule information already earlier and stronger than PFC neurons.
To better understand the cortical network involved in encoding quantitative rules applied to multiple magnitudes, we recorded single-cell activity simultaneously from PFC, dorsal premotor cortex (PMC) and cingulate motor cortex (CMA). Monkeys were trained to switch between 'greater than/less than' rules applied to either spatial or discrete magnitudes (line length or numerosity). In contrast to previous findings, but in accordance with the view that prefrontal cortex is involved in most abstract tasks, we found the most and strongest abstract rule activity in PFC as compared to PMC and CMA. Neurons that generalized the magnitude principle and thus responded abstractly to the overarching concept "magnitude rules" were exclusively present in PFC. In PMC and CMA, however, the rare rule-selective neurons were specialized to a specific magnitude type. This indicates that especially the PFC is an important structure to implement abstract rules related to quantity information.

Disclosures: A. Eiselt: None. **A. Nieder:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.11/JJJ37

Topic: F.02. Animal Cognition and Behavior

Support: National Institute of Mental Health

Title: Neuronal activity in prelimbic and orbitofrontal cortex during operant set shifting in rats

Authors: *A. DEL ARCO[1,2], Y. KIM[1], J. WOOD[1], J. PARK[1], B. MOGHADDAM[1];
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Abstract: Prefrontal cortex plays a critical role in learning and maintaining the rules that guide behavior. Both prelimbic prefrontal cortex (PLPFC) and orbitofrontal cortex (OFC) contribute to flexible behavior, in part, through the assessment of previous behavioral outcomes and choices. To better understand how neuronal activity in PLPFC and OFC contribute to flexible, rule-based behavior across multiple trials, we recorded single unit activity and local field potentials (LFP) in both regions during an operant set shifting task during which animals perform several extra-dimentional shifts between two rules (Darrah et al. Behavioural Pharmacology 19: 225-234, 2008). This task is sensitive to the functional integrity of PLPFC and involves shifting between (1) illumination of a nose poke port, regardless of location, and (2) location of the port (right,

center or left), regardless of illumination. Animals had to adopt the current rule by trial and error, based on the delivery or omission of reward after each trial. Each recording session was completed when the animal successfully performed 3 shifts between 4 sets. Set shifting performance was stable during the recording sessions. Animals required 143 ±10 trials to complete the task, making 38 ±3 total errors. Single unit analysis showed that different proportions of cells in the PLPFC and OFC were activated and inhibited during different events of the set shifting task. The firing rate of a subpopulation of PLPFC and OFC units was significantly modulated by feedback from both the previous trial and the current trial. However, more PLPFC units compared to OFC were associated with the outcome of the previous trial which suggests that PLPFC has a major role in tracking the recent history of rewards. Both prefrontal regions showed an increase in low gamma band LFP power (approximately 40 Hz) spanning the delay from the instrumental poke to the outcome. This increase in gamma power may be critical for local circuit processing of the selected action and involved in linking the choice to the outcome of that choice.

Disclosures: A. Del arco: None. **Y. Kim:** None. **J. Wood:** None. **J. Park:** None. **B. Moghaddam:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.12/JJJ38

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 2R01MH065252-06

Simons Foundation

Richard and Linda Hardy

NSF-DMS-1042134

Title: Enhanced functional connectivity between prefrontal cortex and striatum during associative learning of a categorization task

Authors: *E. G. ANTZOULATOS, R. LOONIS, E. K. MILLER;
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Abstract: The ability to extract the "essence" of diverse experiences is called categorization. It is compromised in autism and schizophrenia and involves a broad network of brain areas, including

the prefrontal cortex (PFC) and striatum. To study the interactions between PFC and striatum, we trained monkeys on a task in which they progressed from simple stimulus-response (S-R) learning through category learning to category performance (Antzoulatos and Miller, 2011). We recorded spiking activity and local field potentials (LFP) from the PFC and striatum simultaneously.

We evaluated changes in the functional connectivity between PFC and striatum across the 3 sequential learning stages described above. Connectivity was assessed as bias-corrected phase synchronization of frequency-transformed LFPs. After the transition from S-R learning to category learning there was an increase in synchronization of PFC and striatal LFPs at the delta (~3 Hz) and beta (~20Hz) bands. This enhancement was primarily observed late in the trial, just before emission of the chosen saccade, consistent with a role in motor preparation. By contrast, changes in early trial PFC-striatal synchronization were observed after the transition from category learning to category performance. These changes were two-fold: First, there was a general decrease in delta-band synchronization. Second, there was an increase in category-specific beta band synchronization, that is, different sets of pairs of PFC-striatal recording sites showed increased beta synchrony for the two different categories. This latter effect was observed for synchronization of prefrontal spikes to striatal LFPs, but not the reverse, i.e. striatal spikes to prefrontal LFPs, suggesting communication from the PFC to striatum. This suggests the emergence of beta-band defined category-specific functional circuits between PFC and striatum. Finally, in contrast to changes in synchronization *between* these 2 areas, there were no comparable changes in synchrony *within* either area and no changes in the spectral power of individual sites. These results give insight into the formation of functional circuits between the PFC and striatum during category learning.

Disclosures: E.G. Antzoulatos: None. **R. Loonis:** None. **E.K. Miller:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.13/JJJ39

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R37-DA006214

NIH Grant R21-DA032005

Title: Orbitofrontal neurons encode reward certainty

Authors: *D. E. MOORMAN, G. ASTON-JONES;
Neurosciences, Med. Univ. of SC, Charleston, SC

Abstract: Accurate execution of complex behaviors is guided by the activity of neural populations across multiple frontal cortical areas. The rodent medial prefrontal cortex (mPFC) has been well-studied with respect to complex behaviors; recent results indicate that neurons in the mPFC encode hierarchical rules that drive task-appropriate responses irrespective of whether that response involves behavioral execution or inhibition. Other frontal regions, in particular the orbitofrontal cortex (OFC), also play an important role in goal-directed behaviors, but the relationship between OFC neuronal activity and response execution or inhibition has been less-well characterized; this is particularly true for extinction learning, an important example of context-appropriate response inhibition. Here, we recorded the activity of OFC neurons while animals performed a discriminative-stimulus (DS)-driven sucrose-seeking task followed by multiple days of extinction of the DS. In contrast to mPFC neurons, OFC neuronal activity was maximally modulated immediately following reward-predicting stimuli (RS) that were followed by a lever press and consumption of sucrose. RSs in the DS-sucrose task that did not produce a lever press, or RSs during extinction, produced comparatively weaker OFC activation, as did non-rewarded stimuli (NS) irrespective of response (press vs. withhold) or session (DS-Sucrose vs. Extinction). OFC neurons were also strongly excited before rewarded well-entry, and were strongly inhibited during reward consumption. Thus, although cue-evoked activity occurred in OFC neurons for all salient stimulus presentations, it preferentially encodes the conjunction of correct reward-prediction (i.e., RS presentation in DS-Sucrose) and behavioral response (i.e., RS-evoked lever press in DS-Sucrose). Although previous work has shown a role for OFC in representing confidence in reward selection, our results extend these findings to demonstrate that outcome confidence appears to be limited to outcomes producing rewards.

Disclosures: D.E. Moorman: None. **G. Aston-Jones:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.14/JJJ40

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 17300103

KAKENHI 20020015

Title: Confidence judgments and prefrontal neuronal activity in monkeys performing a spatial working memory task

Authors: *A. TANAKA[1], S. FUNAHASHI[1,2];
[1]Kokoro Res. Ctr., [2]Grad. Sch. of Human and Envrn. Studies, Kyoto Univ., Kyoto, Japan

Abstract: Over the past years, we have investigated the role of the prefrontal cortex in confidence judgments by macaque monkeys that are engaged in a modified oculomotor delayed-response (ODR) task. In this task, the monkeys are required to remember the location of a visual cue during a several-second delay period, after which they are sometimes forced to take a recognition (matching-to-location) test (forced-test trials) and sometimes allowed to choose to either take or escape from the test (chosen-test or chosen-escape trials, respectively). We previously showed that the monkeys' memory performance was higher in chosen-test trials than in forced-test trials, which suggests that they tended to escape from the memory test when they were likely to make an error. We also reported that spatial selectivity of persistent delay-period activity in prefrontal neurons tended to be lower when the monkeys chose to escape from the memory test than when they made a correct response in the chosen-test or forced-test trials. These results suggest that the spatially selective delay-period activity of prefrontal neurons contributes to the monkeys' confidence judgments about the memory of the cued location. Here we provide additional evidence for this interpretation. Specifically, we examined a small fraction of spatially selective neurons, for which we could collect a sufficient number of error trials (i.e., forced-test and chosen-test trials in which the subject made an incorrect response). For the present analysis, trials were divided into four types: (1) correct forced-test, (2) correct chosen-test, (3) chosen-escape, and (4) error trials. For each trial type, we obtained trial-by-trial firing rates for each neuron's preferred and non-preferred directions, and used them to calculate the area under the receiver operating characteristic curve (AUC) as an index of spatial selectivity. In both cue and delay periods, the AUC values for the correct forced-test and correct chosen-test trials were significantly higher than those in the chosen-escape and error trials, while there was no significant difference between the former two and between the latter two. This trend was particularly pronounced in the late part of the delay period (i.e., immediately before the subject's decision to take or escape from the test). These results support the idea that the monkeys are inclined to escape from the memory test when the risk of error is high and that spatially selective neuronal activity in the prefrontal cortex is a source of confidence judgments in the context of ODR performance.

Disclosures: A. Tanaka: None. **S. Funahashi:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.15/JJJ41

Topic: F.02. Animal Cognition and Behavior

Support: DC 004845

Title: The role of ventral prefrontal cortex in auditory, visual and audiovisual working memory

Authors: *B. PLAKKE[1], J. HWANG[2], M. D. DILTZ[1], L. M. ROMANSKI[1];
[1]Dept. of Neurobio. & Anat., Univ. of Rochester Sch. of Med., Rochester, NY; [2]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: The ventral frontal lobe is well known for its role in language processing and social communication. Previous work has demonstrated that individual neurons within ventrolateral prefrontal cortex (VLPFC) process and integrate face and vocal information (Romanski et al., 2005; Sugihara et al., 2006). We have also recently shown that VLPFC neurons are active during audiovisual working memory (Hwang and Romanski, 2010). The effect of VLPFC lesions on tasks which assess learning or memory has been equivocal, with some studies demonstrating impairment on visual learning and memory, auditory discrimination, rule learning, and decision making, while other studies have failed to demonstrate any deficits during working memory tasks. In the current study we asked whether VLPFC was essential in an audiovisual non-match-to-sample (NMTS) task designed to assess auditory and visual working memory. During the task, rhesus macaques attended an audiovisual movie as the Sample and were required to press a button when a non-matching stimulus, i.e. one whose auditory or visual track differed from the Sample, occurred. Our subjects detected the Non-Match (NM) with a button press in order to receive a juice reward. We inactivated VLPFC while subjects performed the task by cooling the cortical surface of VLPFC below 20° C. During each testing session, 100 baseline trials were completed (WARM trials) and then the cortex was cooled below 20° C, and another 100 trials were completed (COLD trials). We assessed performance accuracy and reaction time in the auditory and visual non-match trials of the audiovisual NMTS task during WARM and COLD trials. Performance accuracy was significantly decreased during COLD trials compared to performance during WARM trials. Subjects also completed trial blocks where only auditory or visual stimulus changes occurred. There was no decrease in accuracy during visual-only Non-Match trials. In contrast, cooling of VLPFC during auditory-only trials resulted in a decrease in performance accuracy. The combined effects across all trial blocks suggests that VLPFC is necessary for crossmodal attention/ switching and auditory working memory but not for visual working memory.

Disclosures: B. Plakke: None. **J. Hwang:** None. **M.D. Diltz:** None. **L.M. Romanski:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.16/JJJ42

Topic: F.02. Animal Cognition and Behavior

Title: The effect of inactivation of dorsolateral prefrontal cortex (DLPFC) in categorical and non-categorical reversal by transcranial magnetic stimulation (TMS) in monkeys

Authors: *T. HOSOKAWA, Y. MATSUI, M. YAMADA, T. IIJIMA, K.-I. TSUTSUI; Tohoku Univ., Sendai, Miyagi, Japan

Abstract: We trained two Japanese monkeys (Macaca fuscata) to perform stimulus-outcome reversal with multiple stimuli. One of eight abstract visual stimuli, half of which were associated with juice and the rest with saline, was chosen to serve as a cue to predict which type of liquid was to be given at the end of the trial. After experiencing each stimulus for 2 or 3 times, monkeys learned the stimulus-outcome relation for all of them, showing anticipatory licking after the presentation of a juice-predicting stimulus, and no licking after the presentation of a saline-predicting stimulus. We occasionally reversed the stimulus-outcome relations without giving any explicit cue. There were two conditions in the reversal. One was the whole reversal condition, in which the stimulus-outcome relation reversed in all stimuli, and the other was the partial reversal condition, in which the stimulus-outcome relation reversed in only 4 stimuli and was preserved in the rest. The monkeys showed quick adaptation to the whole reversal, but they were slower to adapt to the partial reversal. The result suggests that the monkeys used different strategies to perform whole/partial reversals. They formed functional categories of stimuli based on the commonality of outcomes during the new learning, and performed category-outcome reversal to quickly adapt to the whole reversal, whereas in the partial reversal, in which the functional categories were unpreserved, they had to relearn the stimulus-reward associations for each stimulus taking much longer time.
We investigated the role of dorsolateral prefrontal cortex (DLPFC) in this task by using repetitive transcranial magnetic stimulation (rTMS) for reversible brain inactivation. The position for rTMS was determined as 20 mm anterior to the primary motor cortex where TMS elicited hand/finger movements. We applied low-frequency rTMS bilaterally before the monkeys performed the task (twice for each hemisphere at 1 Hz for 5 minutes). When DLPFC was inactivated by the low-frequency rTMS, the monkeys needed significantly more trials to adapt their behavior in the whole (or categorical) reversal, but no change of performance was observed

either in the new learning or partial (or non-categorical) reversal. This result suggests that DLPFC plays a critical role in the top-down behavioral control utilizing category and rule information, but not in bottom-up association learning.

Disclosures: T. Hosokawa: None. **Y. Matsui:** None. **M. Yamada:** None. **T. Iijima:** None. **K. Tsutsui:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.17/JJJ43

Topic: F.02. Animal Cognition and Behavior

Title: Logical reasoning in primates: Monkeys' prefrontal cortex neurons are modulated during manipulation but not during learning of new information

Authors: *F. DI BELLO, V. MIONE, A. GENOVESIO, E. BRUNAMONTI, S. FERRAINA; Dept Human Physiol. & Pharm, Sapienza Univ. of Rome, Rome, Italy

Abstract: One of the most remarkable traits of highly encephalized animals is their ability to flexibly manipulate knowledge in order to infer logical relations. The corresponding cognitive process can be operationally named reasoning. Transitive inference (TI), i.e., the capacity to conclude that A < C, by knowing that A < B and B < C has been used, in different forms, to study logical reasoning and knowledge manipulation in humans and other animals.
We studied the properties of a sample of neurons recorded from the Prefrontal cortex (PFc) of two monkeys trained in a TI task to investigate the underlying neural correlates. Animals were trained to learn, in a single session, the relationship between six adjacent items of an arbitrarily rank-ordered sequence (i.e. A>B; B>C; C>D; D>E; E>F; learning phase) and then to deduct the relationship between novel (non-adjacent, e.g., B>D or C>E) pairs never experienced during the learning phase and presented intermingled to learned pairs (test phase).
Behaviorally, subjects tested in TI tasks display both the symbolic distance and the serial position effects. The symbolic distance effect predicts that the performance improves with the difference between the rank of the items to be compared, e.g. comparing B and E should be easier than comparing B and C, while the serial position effect predicts that at a given symbolic distance, the performance in comparing the items with intermediate ranks is worse than comparing items with extreme ranks.
We found that the activity of about one third of the PFc neurons tested reflected the behavioral effects identified: they were tuned for the serial position effect, the symbolic distance or for both.

Interestingly, the great majority of the neurons displayed a modulation for adjacent pairs comparisons only during the test phase but not during the learning. The present results provide a first evidence of the importance of PFc in reasoning in primates within the information manipulation phase of the logical process involved in TI tasks.

Disclosures: F. Di Bello: None. **V. Mione:** None. **A. Genovesio:** None. **E. Brunamonti:** None. **S. Ferraina:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.18/JJJ44

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI No. 21240024

KAKENHI No. 25240021

Title: Pair selectivity of primate prefrontal neurons in visual paired association performances

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Abstract: The prefrontal cortex (PFC) has been known as an important brain structure for executive control. To accomplish executive control, the PFC requires monitoring the operations in other brain structures and controlling them by sending control signals named “top-down signals.” It has been shown that top-down signals provided from the PFC play important roles to retrieve specific information stored in the posterior cortices as long-term memory. Sakai and Miyashita (1991) showed that top-down signals play a role to produce “pair-recall” activity in inferotemporal (IT) neurons, which reflects retrieval of a paired associate of the sample stimulus while monkeys performed a pair-association task. Although studies indicate that the PFC sends top-down signals to perform executive control, neural correlate of the top-down signal is not yet known. In the present study, we used a paired association task with 12 pairs of visual stimuli to examine neural correlates of top-down signals in the PFC. PFC neurons with visual response exhibited stimulus selectivity and stimulus-pair selectivity. Comparison of these selectivity with IT neurons revealed that PFC neurons had broader stimulus selectivity but had similar values of pair selectivity. Further, PFC neurons with delay-period activity also exhibited stimulus selectivity and stimulus-pair selectivity. Indices showing the strength of stimulus selectivity and

stimulus-pair selectivity were similar between visual activities and delay-period activities. However, the strength of stimulus-pair selectivity of delay-period activity was increased with a progress of the delay period. These results indicate that pair-selective activity observed in PFC neurons could be neural correlates of top-down signals that the PFC provides to the IT cortex during pair association performances.

Disclosures: S. Funahashi: None. **J.M. Andreau:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.19/JJJ45

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH002887

Title: The contribution of the amygdala to stimulus-reward related neural activity within the orbital and medial prefrontal cortex of rhesus macaques during learning

Authors: J. A. RIPPLE, *P. H. RUDEBECK, A. R. MITZ, E. A. MURRAY;
Section on the Neurobio. of Learning and Memory, Lab. of Neuropsychogy, Natl. Inst. of Mental Hlth., BETHESDA, MD

Abstract: The orbital (OFC) and medial (MFC) prefrontal cortex both play critical roles in reward learning. Although it is well established from recording studies in macaques that neurons in OFC and MFC signal changes in reward contingency during learning, the origin of these signals is unclear. The amygdala (AMG), which is reciprocally interconnected with both OFC and MFC, is one of the possible sources of this reward-related activity during learning. Consistent with this idea, AMG neurons, like neurons in the OFC and MFC, signal the contingency between stimuli and rewards during learning. We have previously shown that lesions of the AMG disrupt the ability of monkeys to learn stimulus-reward contingencies (Rudebeck et al., SFN abstract, 2012). To determine whether AMG is critical for the development of stimulus-reward association signals within the OFC and MFC during learning, we recorded neural activity within these areas in three monkeys performing a stimulus-reward learning task both before and after bilateral lesions of the AMG. Within each session, monkeys were given the opportunity to learn about three novel visual stimuli and their associated amounts of fluid reward. On each trial, monkeys were shown a pair of stimuli; choice of a given stimulus led the delivery of fluid in the amount assigned to that stimulus. Through trial and error, monkeys learned to choose the stimulus that led to the greater amount of reward. While monkeys

were engaged in the task, single neuron responses in OFC and MFC were recorded. Task-responsive neurons were classified based on changes in activity in response to a number of task-related variables. These included the amount of reward associated with the two stimuli presented, the order in which the stimuli were presented, position of the stimuli on the screen on each trial and whether the best stimulus was chosen on each trial. For task-responsive neurons, stepwise and best-subsets model-fitting procedures were used to identify variables that best explained neural activity during the task. Best-fitting models were used to compare activity across areas both before and after lesions of AMG. Relative to before the lesion, neurons in OFC showed altered encoding of the choice of stimulus associated with the greater amount of reward. By contrast, relative to before AMG lesions, neurons in MFC displayed an increase in modulation of activity related to the position and value of the chosen stimulus. In combination with the decrement in behavioral learning rate observed following AMG lesions, these changes in neuronal activity suggest a role for AMG in the incorporation of feedback with stimulus-reward value in OFC during learning.

Disclosures: J.A. Ripple: None. **A.R. Mitz:** None. **E.A. Murray:** None. **P.H. Rudebeck:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.20/JJJ46

Topic: F.02. Animal Cognition and Behavior

Support: University of New England Faculty Development Grant

Title: The anterior claustrum and flexible behavior in the rat: A comparison of NMDA and dynorphin-saporin lesions

Authors: *A. C. TALK[1], D. BERNASCONI[1], Z. STEVENS[1], K. GRASBY[1], L. EDELSTEIN[2], J. SMYTHIES[3], B. RUSSELL[4];
[1]Univ. of New England, Armidale, Australia; [2]Medimark Corp., San Diego, CA; [3]Univ. of California, San Diego, CA; [4]Advanced Targeting Systems, San Diego, CA

Abstract: The claustrum is a small structure of poorly understood function situated subcortically in the basal forebrain. The fact that it is extensively and reciprocally connected with the cerebral cortex has led to suggestions that it is involved in coordination of cortical activity. Here we created lesions to the anterior claustrum of rats and tested performance on tasks that involve neural processing in one or more frontal or limbic cortical structures. The excitotoxin NMDA

was used to create partial lesions to the anterior claustrum. Lesions were constrained to the claustrum except in a minority of subjects. Taking into account the fact that some of the highest densities of kappa-opioid receptors in the mammalian brain are in the claustrum, in a separate study we used a custom dynorphin-saporin conjugate supplied by Advanced Targeting Systems to create lesions. We found that the claustral lesions using the targeted immunotoxin could be more complete than those provided by NMDA. In our first study, after excitotoxic lesions created by infusions of NMDA, we tested spatial reversal learning in a water maze. Lesioned rats were not impaired at acquiring the initial location of a goal platform in the maze, but were impaired at acquiring a switched location in the reversal phase. The lesioned rats also exhibited more perseverance errors compared to control rats during reversal. These same rats were not impaired at latent inhibition or working memory tasks, suggesting the effect of anterior claustral lesions may be related to behavioral flexibility. This finding is consistent with theories of claustral function that suggest it may help coordinate information necessary for cortical-dependent tasks. We are currently assessing the role of the anterior claustrum on other measures of behavioral flexibility using a cohort of rats that have been subjected to immunotoxic lesions.

Disclosures: A.C. Talk: None. **D. Bernasconi:** None. **Z. Stevens:** None. **K. Grasby:** None. **L. Edelstein:** None. **J. Smythies:** None. **B. Russell:** None.

Poster

574. **Executive Function: Corticostriatal Mechanisms**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 574.21/JJJ47

Topic: F.02. Animal Cognition and Behavior

Title: A Bayesian model for neural coding

Authors: *S. BEHSETA[1], B. ZHOU[2], D. MOORMAN[4], B. SHAHBABA[3], H. OMBAO[3]; [1]California State Univ., Fullerton, CA; [2]Statistics, [3]Deaprtment of Statistics, Univ. of California at Irvine, Irvine, CA; [4]Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: We propose a flexible nonparametric Bayesian model for identifying dependencies between multiple neurons. More specifically, our model can be used to detect exact and lagged synchrony of a pair of spike trains. Utilizing Gaussian Processes, we propose a flexible framework (i.e., it covers a wide range of functional forms) to model the underlying firing rates. Using simulated data, we show that our approach provides a flexible framework for firing rates, identifies dependent neurons, and estimates lag times correctly. We showcase our model by applying it on data obtained from an experiment whose main objective was to investigate the role

of different prefrontal cortical areas in guiding reward-seeking behavior and inhibition of reward-seeking in the absence of a rewarded outcome.

Disclosures: S. Behseta: None. **B. Zhou:** None. **D. Moorman:** None. **B. Shahbaba:** None. **H. Ombao:** None.

575**Poster**

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.01/JJJ48

Topic: F.02. Animal Cognition and Behavior

Title: Pramipexole disrupts synaptic plasticity the ca1 area of the hippocampus of rats that develop contrafreeloading for water, an animal model of compulsive behavior

Authors: *C. SCHEPISI[1], S. PICCININ[2], S. S. D'ORONZIO[1], A. PIGNATARO[3], M. AMMASSARI-TEULE[3], R. NISTICÒ[2], P. NENCINI[1];
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Abstract: >**INTRODUCTION**
Chronic treatment with the preferring D3 agonist pramipexole (PPX) elicits contrafreeloading (CFL), consisting in a compulsive selection of a suboptimal choice. Functional correlates of this behavior have never been investigated so far. Ventral hippocampus has been implicated in behavioral flexibility and decision-making. Synaptic dysfunctions in this area may be involved in the development of abnormal CFL.
OBJECTIVES
We tested whether the development of PPX-induced CFL is associated with an impairment of functional and structural synaptic plasticity in the CA1 region of the hippocampus.
MATHERIALS AND METHODS
Rats were trained under a fixed ratio 3 (FR3) schedule of reinforcement for water. On days 1-6, water was only available through lever pressing while from day 7 to 20 choice between contingent and non-contingent access to water was allowed. PPX 0.5 mg/kg was administered intraperitoneally just before the beginning of the session. Hippocampal slices from the same animals were used to study bidirectional synaptic plasticity using extracellular recordings. Dendritic spine density was also measured.
RESULTS
PPX-treated rats exhibited a rigid behavior as they continued to work for water even though it

was also non-contingently available, thus enhancing spontaneous CFL. Moreover responding for water was not goal-directed since only a fraction of water gained was actually consumed by PPX treated rats. LTP elicited by a single high-frequency stimulation train (100 Hz, 1 s) was significantly reduced in PPX-treated rats with respect to controls. Moreover, PPX treatment modified dendritic spine density in the CA1 region of the hippocampus.

DISCUSSION

We demonstrated that the enhancement of CFL was associated with impairment of hippocampal synaptic plasticity in PPX-treated rats. As CFL results from a loss of behavioral flexibility and goal orientation, which represent the hallmarks of compulsive behaviors, we may speculate that synaptic dysfunction in this area is relevant for the pathogenesis of compulsive symptoms caused by D3-preferring dopaminergic agents.

Disclosures: C. Schepisi: None. **S. Piccinin:** None. **R. Nisticò:** None. **A. Pignataro:** None. **S.S. D'Oronzio:** None. **P. Nencini:** None. **M. Ammassari-Teule:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.02/JJJ49

Topic: F.02. Animal Cognition and Behavior

Support: Marie Curie Intra-European Fellowship

Fondation Neurodis

Agence Nationale de la Recherche

Fondation de France

Fondation pour la Recherche Médicale

Ministère de l'enseignement et de la Recherche

Title: The early cognitive, neurophysiological, and neuropharmacological effects of a slow MPTP-induced dopaminergic lesion in macaque monkeys prior to motor symptoms

Authors: *C. R. WILSON[1,2], F. M. STOLL[1,2], M. C. M. FARAUT[1,2], J. VEZOLI[1,3], V. LEVIEL[1,2], E. PROCYK[1,2];

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Abstract: We investigated the 'pre-symptomatic' phase and non-motor symptoms of Parkinson's disease (PD) in a rhesus macaque model, using a low-dose protocol of the neurotoxin MPTP.
Two monkeys learned the Problem Solving Task (PST) by searching amongst several targets for one that was associated with juice reward (the search phase, SEA), and then repeating this correct response a number of times (the repetition phase, REP). A change signal then instructed them to begin a new problem by searching again. The contrast between SEA and REP phases provides an index of cognitive control (Procyk & Goldman-Rakic 2006). Monkeys were chronically implanted with at least 22 electrodes resting on the dura mater to provide electroencephalographic (ECoG) recordings.
During a stable control period, we characterized electrophysiological markers in 20-30Hz beta oscillations over frontal cortex during the delay period prior to each trial. Beta power was modulated both by cognitive control demands in the SEA and REP phases of the task, and also by within-session progression towards a final large bonus reward, demonstrating ongoing behavioural adaptation related to time-on-task.
After this control period, monkeys received a slow low-dose MPTP treatment (0.2 mg/kg, max frequency 1 injection/week). After each injection, monkeys received 2 days of rest followed by 5 days of testing with ECoG recordings. Daily analysis included cognitive performance and ECoG activity. Progression of the treatment was also monitored using PET-imaging with the ligand [11C]PE2I in order to follow levels of the dopamine transporter DAT, and scoring of motor symptoms on the Parkinsonian Monkey Rating Scale (PMRS).
Throughout the MPTP phase monkeys remained below the threshold for clinically significant motor symptoms on the PMRS, and so were in the 'pre-symptomatic' phase of the PD model. We observed a general maintenance of beta oscillatory power with ongoing treatment, and this power continued to reflect differences between SEA and REP phases of the task. We did, however, note changes in power between acute periods after an injection of MPTP, and 'recovery' periods without injections, hinting at a possible signal of recuperation. Time-on-task effects remained robust in the face of treatment as well. The maintenance of oscillatory patterns can be seen in the context of a recorded increase in DAT binding potential in the early stage of the lesion measured by the PET-imaging. This ongoing project will provide characterization of early neurophysiological and cognitive alterations induced by a dopaminergic lesion.

Disclosures: C.R. Wilson: None. **F.M. Stoll:** None. **M.C.M. Faraut:** None. **J. Vezoli:** None. **V. Leviel:** None. **E. Procyk:** None.

Poster

575. Decision Making: Behavioral and Pharmacological Studies

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.03/JJJ50-DP10

Topic: F.02. Animal Cognition and Behavior

Support: PHS grant R01-MH092868

Title: Locus coeruleus neuronal activity during stop task performance in rats

Authors: *A. BARI, M. D. RIEDY, G. ASTON-JONES;
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Abstract: Locus coeruleus-norepinephrine (LC-NE) neurons are involved in attention, decision-making and executive control. Impulsivity and attentional deficits are characteristic of pathological conditions such as Parkinson's disease, schizophrenia and attention deficit/hyperactivity disorder (ADHD), all associated with NE dysfunctions. Deficits in attention and response control can be assessed in the laboratory with the stop-signal task (SST), and are ameliorated by drugs that increase brain NE signaling. For example, drugs currently used for the treatment of ADHD (like methylphenidate and atomoxetine) have strong effects on the LC-NE system, and improve executive functions in affected as well as unaffected subjects. Here, we used a modified version of the rodent SST that tracks and adjusts with the animal's performance and is similar to the task version used in human subjects. A double-tracking algorithm adjusts stop signal delays based on the animal's stop accuracy according to a staircase procedure, while also taking into account the speed of the behavioral responses. This procedure yields stable and reliable estimates of the stop-signal reaction time (SSRT), which represents the speed of the underlying inhibitory process. We recorded single and multi unit activity of LC-NE neurons in rats performing the double-tracking SST procedure using microwire electrode assemblies (tetrodes) mounted on a drivable implant. Preliminary results indicate that LC neurons are phasically activated by stop signals that are presented on a subset of trials and instruct the animal to inhibit the ongoing behavior. Phasic activation of LC-NE neurons is larger for successful stop trials (response inhibition) compared with stop errors. During go trials (when no stop signal is presented), LC phasic activity precedes the behavioral response, but only on correct trials. Altogether these results indicate that LC phasic activity differentially signals whether the animal will inhibit or perform a certain action based on task rules and decision processes. These preliminary results confirm the proposed role of the LC-NE system in response inhibition, attention and decision making, and are highly relevant for modern theories of attention and executive control of behavior. Supported by PHS grant R01-MH092868.

Disclosures: A. Bari: None. **M.D. Riedy:** None. **G. Aston-Jones:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.04/JJJ51

Topic: F.02. Animal Cognition and Behavior

Support: NSERC Grant 7861-2010

Title: Effects of acute yohimbine on rat inhibitory control tested with the countermanding paradigm

Authors: *J. BEUK, R. J. BENINGER, E. M. MECHEFSKE, M. PARÉ;
Dept. of Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada

Abstract: The countermanding paradigm assesses inhibitory control by measuring a subject's ability to withhold a response to a go stimulus when a stop signal is presented occasionally (Logan & Cowan, 1984). In rats, administration of yohimbine, an antagonist of alpha-2-adrenoceptors, has been suggested to increase impulsive behaviour in the form of premature responding in the 5-choice serial reaction time test (Sun et al., 2009; Torregrossa et al., 2012). Here, we studied the exact contribution of noradrenergic receptor activation to inhibitory control by examining the effect of acute yohimbine treatment on rat performance in a countermanding task. Increased impulsivity can be demonstrated in the countermanding task by faster responding and slower stopping. Male Wistar rats (N = 21) were trained to respond to a visual stimulus (go signal) by pressing a lever below an illuminated light for food reward, but to countermand the lever press (25% of trials) subsequent to an auditory tone (stop signal) presented after a variable delay. Rats were randomly administered yohimbine (0, 1.25, 2.5 mg/kg; i.p.) immediately prior to 60-min test sessions. Yohimbine treatment significantly increased the number of trials rats performed in a session, and it decreased both response time and the proportion of go trial errors. Stopping ability was affected by yohimbine dependent on baseline performance; faster stopping rats were more impaired (slower) following yohimbine administration while slower stopping rats demonstrated improved (faster) stopping. These results suggest that yohimbine may increase impulsive behaviour by both speeding 'go' processes and impairing 'stop' processes in usually fast-stopping animals. (Funded by NSERC)

Disclosures: J. Beuk: None. **R.J. Beninger:** None. **E.M. Mechefske:** None. **M. Paré:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.05/JJJ52

Topic: F.02. Animal Cognition and Behavior

Support: R21 MH097067

Title: Behavioral and neurochemical characterization of mutant mice lacking Lphn3, a gene implicated in ADHD and addiction

Authors: *C. A. ORSINI[1], D. WALLIS[2], B. SETLOW[1];
[1]UNIVERSITY OF FLORIDA, GAINESVILLE, FL; [2]TEXAS A&M UNIVERSITY, COLLEGE STATION, TX

Abstract: The latrophilin 3 (*Lphn3*) gene has been linked to susceptibility to attention deficit hyperactivity disorder (ADHD) and vulnerability to development of addiction. Specifically, several groups have used gene linkage and association studies to demonstrate that ADHD and substance abuse are both linked to chromosome 4 in the area of the *Lphn3* gene. This suggests that this gene may be a genetic biomarker of these disorders and may lead to more selective therapeutic targets for treating them. However, little is known about the function of this gene and its protein product. To characterize the function of the *Lphn3* gene, we generated mutant mice for the *Lphn3* gene using a gene-trap embryonic stem cell line. We then performed neurochemical, pharmacological, and behavioral assays to assess *Lphn3* function. In one cohort of mice, we harvested tissue from 4 to 6 week old male mice to quantify levels of dopamine and serotonin in the dorsal striatum. Intriguingly, we observed that *Lphn3* mutant mice had significantly higher dopamine and serotonin levels than their wild type (WT) counterparts. Given that elevated striatal dopamine is associated with locomotor hyperactivity in other mouse mutant lines, another cohort of male and female mice were evaluated in an open field arena to determine if there were differences in locomotor activity. As expected, *Lphn3* mutant mice displayed significantly more horizontal activity than both WT and heterozygous mice. Additionally, when *Lphn3* mutant mice were administered acute i.p. injections of cocaine (20 mg/kg), they showed a significant elevation in locomotor activity relative to WT mice. Finally, in a separate cohort of male and female mice, we explored the contribution of the *Lphn3* gene to reward-seeking behavior by assessing instrumental responding (lever pressing) for food pellet rewards under various fixed ratio (FR) schedules of reinforcement. Mice were first trained on a FR1 schedule for five 30 min sessions, after which they were tested on FR3, FR10, FR20 and FR40 schedules (one schedule/session). *Lphn3* mutant mice displayed significantly greater instrumental responding for food than WT mice, particularly under high response ratios. Together, these findings are consistent with a role for *Lphn3* in regulating motivated behavior, and show that a loss of gene function results in increased reward-seeking, possibly via enhanced striatal monoamine signaling. Current work is focused on characterizing *Lphn3* mutant mice in behaviors more closely linked to ADHD, such as impulsivity, and investigating the

neurobiological consequences of the *Lphn3* mutation.
Research is supported by R21 MH097067 (NIMH) to D. Wallis and B. Setlow

Disclosures: C.A. Orsini: None. **D. Wallis:** None. **B. Setlow:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.06/JJJ53

Topic: F.02. Animal Cognition and Behavior

Support: NSF 1121147 to ML

Title: Population coding of decision contexts by ensembles of striatal neurons

Authors: *M. LAUBACH[1,2], J. COCKBURN[3], E. Y. KIMCHI[4], M. J. FRANK[3];
[1]Yale Univ., New Haven, CT; [2]John B. Pierce Lab., New Haven, CT; [3]Cognitive, Linguistic & Psychological Sci., Brown Univ., Providence, RI; [4]Neurol., Brigham and Women's Hosp., Boston, MA

Abstract: Decisions are not made in isolation but in specific contexts and as part of sequences. The neuronal basis of integrating information about previous behavioral decisions into the current decision context has not been resolved. The striatum is a key structure for decision making and has been shown by several groups to contain neurons that encode prior actions and outcomes. Here, we studied the potential role of the striatum in context-based decision making using a task (Kimchi and Laubach, 2009) in which the reward value of a stimulus depends on the stimulus set. A computational analysis of the task found that changes in action selection in this task can be resolved by a one-back memory of the stimulus and action from the previous trial. Reinforcement learning models were developed for the task and were fit to rats' behavioral performance. Learning models that operated on a state space (defined by the conjunctions of the current and previous stimuli and the previous action) learned to rapidly adjust behavior in line with changes in the stimulus set, as was observed in animal behavior. Most recently, we used a predictive modeling approach (aka decoding, MVPA) to validate these models in terms of trial-to-trial fluctuations in spike activity in the striatum. We found that (1) contextual states and state-action values from the conjunctive state-space model can be decoded significantly better than expected by chance by using the collective activity of ensembles of neurons, but not by using the activity of single neurons, (2) decoding was based on sparse distributed patterns of ensemble activity, with cells “tuned” to specific conjunctions of events from the previous trial, and (3) single neurons only partially predicted the model states, weakly predicted changes in the state-

action values, and contributed redundant information to the population code. Our findings suggest that decision contexts are defined by precise patterns of spike activity that are distributed over groups of striatal neurons and that action values are updated through coordinated changes in these activity patterns.

Disclosures: M. Laubach: None. **J. Cockburn:** None. **M.J. Frank:** None. **E.Y. Kimchi:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.07/JJJ54

Topic: F.02. Animal Cognition and Behavior

Title: Hierarchical population coding of trial phases by the striatal neurons during a choice task

Authors: *M. ITO, K. DOYA;
Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: The striatum is a major input site of the basal ganglia that takes an essential role in decision making. Recent imaging and lesion studies have suggested that the subareas of the striatum have distinct roles.
We recorded neuronal activities from the dorsolateral striatum (DLS, n=190), the dorsomedial striatum (DMS, n=105), and the ventral striatum (VS, n=119) of rats performing a choice task. In this task, when a rat poked into the center hole, one of three cue tones was presented. The rat was required to keep nose-poking during the tone presentation and then to poke either the left or right hole after the offset of the tone. A food pellet was delivered probabilistically depending on the presented tone and the selected poking.
Our previous analysis found that each striatal neuron had a distinct activity pattern (multiple activity peaks through a trial), and that the firing rates at those peaks were modulated by the state (cue tones), the chosen action, the acquired reward, and the reward probability. Here we consider a hypothesis that the variety of activity patterns of a population of striatal neurons encode which phase of what trial a rat is currently in, which can be used for reward prediction, action selection, and learning.
The recorded neuronal spikes in all trials were piece-wise linearly mapped to a standard trial duration of 8.0s so that the times of key task events (the onsets and offsets of the nose pokings and the cue tones) match those of the standard trial. Seven trial epochs were defined as the periods between neighboring task events. Each trial epoch was divided into 4 to 20 trial phases of 100ms time period. One trial consists of 80 trial phases.

We randomly sampled 100 neurons from each subarea and estimated the trial phases from their population activities by a non-parametric Bayesian method. The prediction accuracy was evaluated for a new data set which was not used to make a prediction model (cross validation). In all subareas, the predictions of all 80 trial phases were significantly higher than the chance level (1/80 = 0.0125). While the average of the prediction accuracy of trial phases was the highest in DLS (DLS: 0.19, DMS: 0.16, VS: 0.10), the average of the prediction accuracy of the trial epochs was the highest in DMS (DLS: 0.71, DMS: 0.76, VS: 0.66). In VS, only the prediction of the reward epoch, after the left- or right- nose poking, is the best in the subareas. These results support our hypothesis that activity patterns of striatal neurons encode the trial epochs with population coding. Increasingly more accurate coding of finer trial phases in VS, DMS, and DLS suggests a hierarchical representation of the progress of a trial in the striatum.

Disclosures: M. Ito: None. **K. Doya:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.08/JJJ55

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 5-R37-MH087027

Title: Varying levels of craniotopy and retinotopy in SEF during a sequential saccade task

Authors: *M. R. SILVER, E. K. MILLER;
Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: The frontal cortices have long been thought to utilize topographic maps to support visuospatial behavior. Retinotopic maps are relative to the eye and thus are gaze-dependent. Craniotopic or head-centered maps are stable during eye movements and may be more ideal for storing and executing oculomotor sequences (Silver et al., 2012). Craniotopic information has been reported in the parietal cortex in the form of gain fields, but the picture in the frontal cortex is less clear because in most animal studies of sequential eye movements the two topographies are confounded. We designed an experiment that eliminates this confound and examined the supplementary eye fields (SEF), a region critical for planning sequential saccades in which both retinotopy and craniotopy have been claimed.
In a variant of the double-saccade task, we trained rhesus macaque monkeys to fixate through the presentation of two spatial cues and a one second delay, and to saccade in order to the two cued locations upon fixation offset. Importantly, the fixation cue could appear at the center of the

screen or at any one of the six other locations. This allows us to differentiate between the variance in neural activity explained by retinotopy and craniotopy.
Both retinotopic and craniotopic information contributed to patterns of neural activity in an intermingled and dynamic fashion. The strength of these factors varied between neurons and also within individual neurons during different phases of the trial. Overall, retinotopic information predominated through the cue and delay intervals, but craniotopy became more prominent during saccade execution. Our results suggest that attempts to characterize activity in frontal cortices according to a single, static topographic mapping may obscure the important role of small-timescale variations in neural representation.
Silver MR, Grossberg S, Bullock D, Histed MH, Miller EK (2012) A neural model of sequential movement planning and control of eye movements: Item-Order-Rank working memory and saccade selection by the supplementary eye fields. Neural Networks, 26, 29-58.

Disclosures: M.R. Silver: None. **E.K. Miller:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.09/JJJ56

Topic: F.02. Animal Cognition and Behavior

Support: Scientific Research on Innovative Areas from MEXT

Grant-in-Aids for Young Scientists (A)

Title: Contrastive roles of the two visual thalamic regions in perceptual choices

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Abstract: It has been often suggested that the thalamo-cortical complex underlies much of our perceptual experience. However, it remains elusive how the thalamus works for perceptual experience. The primate visual thalamus has two regions: the lateral geniculate nucleus (LGN) and the pulvinar (Pul), the largest thalamic area, which has interconnections with the multiple visual cortices. To explore the functional role of visual thalamus in subjective visual experience, we recorded the single-unit activities in the visual thalamus while the monkeys performed a perceptual categorization task and an opt-out task. In addition, we inactivated the unilateral visual thalamus by injecting the GABA receptor agonist (muscimol) to examine causal roles of Pul and LGN in the two tasks.

In the perceptual categorization task, the responses of many Pul neurons showed no specificity to any perceptual contents, but their magnitude decreased in a graded manner as the stimulus ambiguity increased. In the opt-out task, the Pul responses to the identical stimuli decreased when the monkeys chose the safe option, indicating less confidence of the animals in their own perceptual categorization. The LGN neurons did not show response modulations in the two tasks. Pharmacological inactivation of Pul increased the monkeys' safe choices in the opt-out task without affecting accuracy in the perceptual categorization task. We observed the effects of pulvinar inactivation (the right side) when the target stimulus was presented in the monkey in the contralateral (left side) visual field but not when the stimulus was presented in the ipsilateral visual field. In contrast, inactivation of the LGN impaired performance on the categorization task and increased the frequency of the safe choice in the opt-out task. These results accord with the view that the LGN (first-order visual thalamus) acts upstream of perceptual categorization whereas the pulvinar (higher-order visual thalamus) acts downstream of visual categorization and plays an essential role in confidence levels of visual categorization.

Disclosures: A. Nikkuni: None. **A. Miyamoto:** None. **K. Numata:** None. **Y. Komura:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.10/JJJ57

Topic: F.02. Animal Cognition and Behavior

Support: 1R01NS079518

Boettcher Foundation

CU Optogenetics Pilot Program

Title: Activity in the mouse pedunculopontine tegmental nucleus reflects trial history

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Abstract: Recent studies across several mammalian species have revealed a distributed network of cortical and subcortical brain regions responsible for sensorimotor decision making. However, most studies have examined relatively simple sensorimotor transformations, whereas real-world decisions incorporate current sensory information and previously acquired knowledge. Few

studies have examined how the brain integrates these two inputs in order to select an action. One area hypothesized to contribute to the representation of actions and their outcomes (i.e., rewarded or non-rewarded) is the pedunculopontine tegmental nucleus (PPTg), in the brainstem. Intriguingly, our recordings in the PPTg of mice engaged in an odor-cued forced-choice spatial task show a subset of neurons that exhibit selectivity for the selected action, or its outcome, on the previous trial. This result is surprising given that the rewarded action on any given trial is independent of trial history, and raises questions about the mouse's strategy on even such a simple task.
We therefore used linear filtering and logistic regression techniques to explore the influence of previous trial factors on the mouse's strategy, and found that choices were indeed influenced by trial history. Notably, on trials in which the odor stimulus was strongly associated with a rewarded action (i.e., "easy" trials),, mice based their choices exclusively on the odor. However, on trials in which the odor stimulus provided little evidence about the correct action (i.e., "hard" trials), mice based their choices more strongly on the actions and outcomes of previous trials. These results suggest that the representations of previous trial history in PPTg, among other regions, may be used to guide action selection.

Disclosures: J.D. Costabile: None. **J.A. Thompson:** None. **G. Felsen:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.11/JJJ58

Topic: F.02. Animal Cognition and Behavior

Support: Marie Curie Intra European Fellowship

Fondation Neurodis

Lyon1 Claude Bernard University

Title: From learning-set to task-set in macaque monkeys

Authors: *M. C. FARAUT[1,2], E. PROCYK[1,2], C. R. E. WILSON[1,2];
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Abstract: Our open-ended and volatile environment requires us to behave in a flexible manner to adapt optimally when contingencies change. Primates can adapt behaviour flexibly in this

way, but questions remain about how this flexibility is implemented, and what the neural correlates of such processes are.
Here we present a project to investigate behavioural flexibility in macaque monkeys, using a test of their ability to adapt to changing task information in an environment that doesn't always give the right answer.
We trained 3 monkeys on a task of Task-Set Manipulation, a monkey equivalent of the task of Collins & Koechlin (2012). In the training phases of the task, monkeys learned by trial and error the association between 2 stimuli and 3 targets using feedback to adapt their responses. Once they reached a behavioural criterion, 2 new stimuli were presented and monkeys entered a new exploration phase. Monkeys thus moved between phases of exploration and exploitation of the stimulus-reward environment. A stochastic reward environment was created by giving invalid feedback to monkeys in 10% of trials. This means that transitions between exploration and exploitation can only be triggered by a continuous checking of environmental information, and not solely by a single feedback, promoting flexibility of behaviour.
We show that monkeys used different learning strategies and adapted their responses to the stochastic environment. Monkeys demonstrated the formation of a stable learning set, a strategy that allows efficient learning of problems, acquired over a number of sessions. For example, over 400 problems, monkey P's performance showed a reduction of mean errors to criterion of 93% between the first 50 and the last 50 problems. This task contrasts with traditional learning-set paradigms which use deterministic 2-choices tasks.
In the final task, for a given session the stimuli no longer changed when the monkey reached criterion, but rather the association between stimuli and targets changed. Thus, the monkey had to switch between these different mappings. We show that monkeys exhibited a level of transfer of their learning-set to the final task. Two types of sessions alternated, some with the same mappings repeated during the session and others with mappings presented only once. The formation of a task-set would be shown by monkeys learning mappings they had already encountered more efficiently than new ones, so with better performance on 'repeated' sessions. We show the effect of the presentation of 'repeated' versus 'one-time' sessions in order to test the ability of monkeys to acquire and use task-sets.

Disclosures: M.C. Faraut: None. **E. Procyk:** None. **C.R.E. Wilson:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.12/JJJ59

Topic: F.02. Animal Cognition and Behavior

Title: Information and value influences on foraging decisions

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Abstract: While most research into the neural mechanisms of decision making focuses on learning reward contingencies for maximizing expected value, animals often already know the expected size and usual location of rewards. Nonetheless, when foraging, animals have to choose which location to visit and learn the spatial distribution of rewards. Rewards harvested while foraging carry information, defined as a reduction in entropy, about the state of the environment, useful for guiding future actions. In a well-learned environment, even after few rewards, the animal may anticipate the information to be gleaned from future actions, which we call expected information. We hypothesize that animals foraging in a well-learned environment maximize their expected information, and, as a corollary, that animals will encode value and information separately. To begin to test this hypothesis, we investigated the neural encoding of information independently of received reward. In our experiment, fixed rewards were randomly assigned to locations in the environment, though not every location was rewarding on every trial. The subject had to select all locations regardless of received reward in order to advance to the next trial. On every trial, the amount of information, operationalized as the reduction in the number of possible spatial allocations of rewards to targets, about the animal's particular environment on that trial decreased over the course of the trial, while the expected value of upcoming actions could decrease or increase, depending on the rewards received so far in the trial.
Response times within a trial are significantly influenced (3,491 trials, 20,946 choices, multilinear regression, all $p < 0.001$) by the amount of information gained from the current choice, by the expected value of the current choice, and by the interaction of the choice number in the sequence with the information gained, choice number with the expected value, and the interaction of expected value and information. Cells in the posterior cingulate cortex (CGp), an area known to be involved in strategic choice, policy selection and adaptive behavior at longer time constants, track the amount of information gained from the current choice (GLM, log-linear link function, $p < 0.05$: 10/11 cells), the expected value of the current choice (5/11 cells), and the interaction of information and expected value (4/11 cells). Our data suggest that information and value both affect an animal's choices when exploring an environment with an unknown spatial distribution of rewards, and that CGp cells encode both value and information, possibly serving as a substrate for the influence of both on decision behavior.

Disclosures: D.L. Barack: None. **J. Gariepy:** None. **M.L. Platt:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.13/JJJ60

Topic: F.02. Animal Cognition and Behavior

Support: NSF Graduate Research Fellowship Program

Title: An oculomotor task for testing metacognitive monitoring of rule selection in monkeys

Authors: *Z. ABZUG, M. A. SOMMER;
Duke Univ., Durham, NC

Abstract: Using rules to guide decisions is an integral part of human cognition. These rules may be instructed or self-selected. The latter case is metacognitive in that a subject must monitor its selection of a rule in order to use it to control future decisions. Prior work showed that monkeys can apply instructed rules to decision-making tasks, and the neuronal basis of that process has been studied. Here we asked, can monkeys select their own rules to apply to future decisions? We designed a two-stage task to probe this question. Both stages involved a two-alternative forced choice. In the first, "rule selection" stage of each trial, the monkey foveated a central spot and two peripheral targets appeared. The monkey was required to saccade to one of the targets. During training, the monkeys learned to associate different colored targets with different abstract rules. The peripheral targets might be of different colors, so that the monkey had to select one of two potential rules; this was the Self-Selected (SS) task condition. Or, the targets might be the same color, so that the monkey had no choice of rule; this was the Instructed (INS) condition. After the monkey made a saccade to one target, the second, "rule implementation" stage of the trial began. The monkey re-fixated the central spot and two complex visual objects appeared in the periphery. They differed from each other in multiple ways (e.g. contrast) corresponding to the rules learnt during training (e.g. "choose the target with lower contrast"). If the monkey applied the rule that was in effect by making a saccade to the rule-appropriate target, it received reward. In one monkey, we found that for both SS and INS trials, and across all rules, performance was significantly greater than chance (t-test, Bonferroni-Holm corrected, $p < 0.05$). The monkey was better at using one rule than the other (ANOVA, $p < 0.003$), with a significant correlation between the likelihood of selecting the preferred rule during SS trials and performance using that rule (Pearson's $r = 0.80$, $p = 0.033$). Our results demonstrate that monkeys can select a rule and implement it for decision-making, an example of metacognitive monitoring and control. We are now using this task to examine neuronal activity in frontal cortical areas during the inter-stage period of SS trials in which the self-selected rule must be remembered for application to behavior later.

Disclosures: Z. Abzug: None. **M.A. Sommer:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.14/JJJ61

Topic: F.02. Animal Cognition and Behavior

Support: HHMI 52005873

PHS Grant 025008

Children's Healthcare of Atlanta

NARSAD

NIH P51OD11132

Title: Social isolation during adolescence but not adulthood modifies outcome-based decision-making

Authors: *E. A. HINTON[1,2], S. L. GOURLEY[3,2];
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Abstract: Background: Adolescence is a vulnerability period for the development of treatment-resistant depression, which includes symptoms such as diminished goal-directed decision-making and anhedonia. Adolescence is also a period of marked structural maturation and refinement in the prefrontal cortex. While contemporary models argue that adolescent-onset psychiatric vulnerabilities emerge in part due to the impact of pathological stimuli on prefrontal cortical development, empirical support remains limited.
Methods: We developed an ethologically-based animal model of adolescent adversity in which female mice are isolated for the majority of the adolescent period and then re-housed in large social groups in adulthood. Decision-making strategies were then classified using response-outcome contingency degradation. In order to examine dendritic spine morphology, we isolated or socially-housed adolescent transgenic mice expressing thy1-derived Green Fluorescent Protein and imaged deep-layer prefrontal cortical dendritic spines.
Results: Adult mice with a history of adolescent-, but not late-adolescent- or adult-, onset social isolation develop stimulus-response habits at the expense of engaging in response-outcome goal-directed decision-making. In addition, mice isolated in adolescence exhibit anhedonic-like insensitivity to sucrose despite being socially re-integrated in adulthood. Our initial evidence also indicates that adolescent isolation fundamentally redirects the developmental trajectory of dendritic spine maturation in deep-layer prefrontal cortex. Adult-emergent behavioral deficits

can be reversed by application of a Rho-kinase (ROCKII) inhibitor during adolescence. Discussion: Given that ROCKII inhibition facilitates activity-dependent cytoskeletal reorganization, our findings provide direct evidence that structural plasticity during adolescence determines long-term behavioral outcomes. Ultimately, improved treatment approaches initiated during the structurally labile adolescent period may have long-term beneficial consequences.

Disclosures: E.A. Hinton: None. **S.L. Gourley:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.15/JJJ62

Topic: F.02. Animal Cognition and Behavior

Title: Changes in behavior and lateral intraparietal neuron activity reveal diverse adjustments in decision-making processes after errors

Authors: *B. PURCELL, R. KIANI;
New York Univ., New York, NY

Abstract: Humans and non-human primates adjust their behavior following errors. These adjustments are thought to reflect strategic changes in decision-making processes to prevent the occurrence of future errors and maximize the rate of reward. Different models propose different mechanisms for post-error performance changes, but these alternative accounts have been difficult to distinguish using behavioral data alone. We investigated the behavioral and neural mechanisms of post-error performance changes in humans and monkeys during a perceptual decision-making task. Subjects viewed a patch of stochastic moving dots and made decisions about the net direction of motion. They reported their choices when ready by making a saccadic eye movement to one of two possible targets. Task difficulty was manipulated by varying the percentage of coherently moving dots. Humans and monkeys exhibited a diversity of behavioral adjustments following errors, with the most common being increased reaction times (i.e., post-error slowing). We fit the subject's behavior with a drift-diffusion model. The model assumes that noisy evidence about motion direction is integrated over time until a response threshold is reached. We found that different subjects employed different mechanisms of post-error adjustments that included delayed integration of evidence, changes in integration rate, and changes of response threshold. We analyzed single-unit responses recorded from the lateral intraparietal area (LIP) of four monkeys to investigate the neural mechanisms underlying these adjustments. The changes in LIP neuron firing rates support our model-based inference about the

differences in the monkeys' decision-making strategies following errors. These results suggest that there is no single mechanism of post-error slowing; rather, different subjects react differently to errors with varying effects on the speed and accuracy of consecutive decisions.

Disclosures: B. Purcell: None. **R. Kiani:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.16/JJJ63

Topic: F.02. Animal Cognition and Behavior

Support: CFI 14033

NSERC 341600

Title: Spatial working memory impairment following selective lesions of the thalamic reuniens

Authors: *J. A. PRASAD, Y. CHUDASAMA;
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Abstract: On account of it's efferent projections to the hippocampus, recent animal studies have emphasized an important role for the midline thalamic nucleus reuniens (NRe) in the long-term consolidation of spatial memory (Loureiro et al., J. Neuro., 32: 9947). By virtue of it's reciprocal connections with the prefrontal cortex, we have shown that the NRe is involved in certain aspects of executive function (Prasad et al, 2013, Brain Struct Funct. 218:85). In addition to cognitive control operations of response inhibition that enable adaptation to a changing environment, efficient executive control requires the capacity to hold information on-line in working memory, and use it to guide on-going behavior. Surprisingly however, we recently showed that selective NRe lesions fail to disrupt performance in a working memory task modeled on the operant delayed response paradigm (Chudasama and Prasad, 2012, SFN Abstr. #599.07). Contrary to our negative result, other studies have shown that lesions or inactivations of the midline thalamus that include the NRe have detrimental effects on spatial tests of working memory (e.g., Hembrook and Mair, 2011, Hippocampus, 21:815). One possibility is that the NRe is sensitive to spatial contexts, in which case its ablation will impair working memory performance in tasks that rely on the use of spatial cues. To test this hypothesis, we tested rats with NRe lesions on a standard win-shift working memory task in the 8-arm radial maze. During the training phase, four of the eight arms were 'closed' and rats were allowed to retrieve pellets from the four 'open' arms. In the test phase (after a 0-second delay), the remaining four arms were opened. Food

pellets were only available if the rat entered a previously 'closed' arm. Unlike sham controls, rats with NRe lesions made several re-entries into the previously 'open' arms, although they were no longer baited [sham: 5.36±1.25; NRe: 9.95±0.99; $p<0.01$]. In contrast, however, the same NRe-lesioned rats showed normal performance in reversal learning and delay discounting tasks. These data therefore suggest that thalamic NRe lesions impair spatial working memory but not other aspects of prefrontal executive function.

Disclosures: J.A. Prasad: None. **Y. Chudasama:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.17/JJJ64

Topic: F.02. Animal Cognition and Behavior

Support: CFI 14033

CIHR 102507

Title: Functional disconnection of the ventral hippocampus and nucleus accumbens affects decision-making

Authors: *A. R. ABELA, Y. CHUDASAMA;
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Abstract: Recently, we showed that rats with ventral hippocampal (vHC) lesions are intolerant to reward delay, and will opt more often for a small immediate reward, instead of a large delayed alternative (Abela and Chudasama, 2013, EJN, 37:640). Rats with lesions of the nucleus accumbens (NAcc) are also intolerant of delay (Cardinal et al. 2001; Science, 292:2499), suggesting that these two structures are concurrently engaged in the assessment of decision costs associated with delay. In line with this speculation, the NAcc receives a direct, excitatory projection from the vHC (Groenewegen et al., 1987, Neurosci, 23:103). However, unlike the vHC, the NAcc is sensitive to the effects of reward uncertainty thereby biasing choices that lead to safe small rewards, rather than large uncertain rewards. Accordingly, in line with optimal choice behavior, rats with vHC lesions and sham controls, reduce their choice of large rewards as the probability of receiving it is decreased, whereas rats with NAcc lesions choose the large reward option significantly less suggesting they are 'risk averse' (Cardinal and Howes, 2005, BMC Neurosci, 6:37; Abela and Chudasama, 2013, EJN, 37:640). This disparity prompted us to examine the interdependent function of the vHC and NAcc in decision making using a

disconnection lesion approach. Rats received unilateral lesions of both the NAcc and vHC, either in the same hemisphere ("ipsilateral") or in opposite hemispheres ("disconnection"), or sham control surgery. Using a touchscreen testing method, rats made choices on two decision-making tasks. In the delay discounting task, rats chose between two visual stimuli, where one stimulus delivered a small, immediate reward and the other a large delayed reward. The delay to the large reward was progressively increased from 0 to 8, 16 and 32 seconds within a session. In the probability discounting task, a new pair of visual stimuli indicated instead a small, certain reward or a large, uncertain reward. The probability of receiving the large reward was systematically reduced from 1 to 1/3, 1/9 and 1/15 every 10 sessions. The results show that all groups of animals preferred the large reward when it was delivered immediately, and shifted their preference to the small, immediate reward as the delay to the large reward increased. However, the group of animals with the disconnection lesion exhibited higher rates of discounting than the other groups. Furthermore, none of the groups were affected by changes in reward uncertainty. These results demonstrate the conjoint importance of the vHC and NAcc in decision making with a delayed outcome.

Disclosures: A.R. Abela: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CFI 14033, CIHR 102507. **Y. Chudasama:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CFI 14033, CIHR 102507.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.18/JJJ65

Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-102752

CIHR MOP-102507

CFI - 14033

Title: Cognitive dysfunction in a transgenic rat model of Alzheimer disease before and after amyloid beta plaque deposition

Authors: E. N. WILSON JR[1], A. R. ABELA[2], V. KNIGHT[2], Y. CHUDASAMA[2], *A. CUELLO[1];
[1]Dept Pharmacol & Therapeut., [2]Psychology, McGill Univ., Montreal, QC, Canada

Abstract: We have developed and characterized a transgenic rat model that recapitulates amyloid beta neuropathology observed in individuals with Alzheimer disease (Leon et al. 2010, J Alzheimer's Disease, 20:113). In these rats, soluble, intracellular amyloid beta accumulates as early as two weeks after birth. Following a conversion to fibrillar form, amyloid beta deposits as extracellular plaques at 6 months of age in the subiculum, and deposition spreads from the dorsal to the ventral hippocampus, and throughout the prefrontal cortex. Our initial studies found that both oligomeric and fibrillar amyloid beta in the hippocampus and entorhinal cortex negatively affect performance on a spatial memory task. Further to it we are interested in how this hippocampal neuropathology affects cognition mediated by hippocampus-prefrontal cortical circuits important in guiding executive function. Accordingly, we examined the performance of Alzheimer transgenic rats with their wild type controls on both one-pair visual discrimination and reversal leaning tasks. In these tasks, rats were required to learn a stimulus-reward association by indicating responses via nose-poke to touch sensitive screens. Separate groups of rats that were either three months old (i.e., before after plaque deposition) or 8 months old (i.e., after plaque deposition) were evaluated on their ability to acquire the stimulus-reward contingency. At the post-plaque stage, relative to their wild type controls, the transgenic rats with Alzheimer-like amyloid beta neuropathology required many training sessions and committed many errors to reach criterion, but were eventually able to acquire the stimulus-reward contingency. In contrast, the transgenic rats at the pre-plaque stage were severely impaired, and were unable to acquire the stimulus-reward association. This difference is likely due to the time-dependent progressive accumulation of toxic amyloid beta oligomers including their deposition as amyloid plaques.

Disclosures: E.N. Wilson Jr: None. **A.R. Abela:** None. **Y. Chudasama:** None. **A. Cuello:** None. **V. Knight:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.19/JJJ66

Topic: F.02. Animal Cognition and Behavior

Support: ECS-0702057

ECS-1002391

Title: What's different in fast and slow learning rats on a directional control task?

Authors: *B. CHENG, Y. YUAN, A. SPINRAD, E. HERRING, J. SI;
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Abstract: Individuals differ when dealing with decision uncertainties, developing elaborative strategies during learning a new task or skill.
The present study used a rat model to reveal neural correlates underlying fast or slow learning of a directional control task. Eight male Long-evans rats at the same age were studies. They were handled and trained to become familiar with paddle pressing prior to learning the directional control task. The task required a rat to choose and press one of the two directional control paddles in response to a respective directional light cue - left cue with a left control press and vice versa. Sugar pellets were delivered after successful trials. The rats were originally naïve and achieved an average behavioral accuracy of 80% in about 30 sessions. Rat's behavior parameters were recorded on video, synchronized with the rat's single unit neural activities from the PM and M1 areas. The rat's physical movement appeared rather stereotypical accompanied with rather consistent reaction time.
The eight recorded rats were divided into fast and slow learning groups. Rats in the fast group usually reached 60% or higher performance accuracy around the 15th session. The remaining four rats are considered slow rats. A pre-learning stage refers to the first several sessions in which the rats' performance accuracies fluctuated between 20% and 70% without displaying a clear and steady upward trend; the learning stage corresponded with the period when the rat's behavioral performance accuracy steadily increased before they reached 80%.
The rats' behavioral performance accuracies and the rats' neural activities were analyzed. During the pre-learning stage, the fast learning rats (dubbed FP rats for fast learning during pre-learning stage) exhibited strong behavioral adaptation after committing an error. The rats were more likely to complete a successful trial after a failure rather than after a success; the post-error accuracy R_es was compared with R_ss using paired t test, $p<0.05$, while no such strong adaptation or only weak adaptation was found for the slow rats (SP rats). During the learning stage, both FL (fast learning during learning stage) and SL (slow learning during learning stage) rats exhibited strong behavioral adaptation. A smaller fraction of neurons (23%) encoded the previous trial outcome for SP rats as compared to the SL (52%), FP (48%), and FL (55%) rats. Taken together, the fast and slow rats differed the most during the pre-learning stage. The fast rats behaviorally adapted quicker post-error trials and a larger fraction of neurons encoded previous trial outcome than slower rats.

Disclosures: B. Cheng: None. **Y. Yuan:** None. **A. Spinrad:** None. **E. Herring:** None. **J. Si:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.20/JJJ67

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB 04-47358

Title: Neuronal circuitry for cost-benefit decision in foraging in Pleurobranchaea and its agent-based simulation

Authors: *R. GILLETTE[1], K. TIAN[2], N. RYCKMAN[2], J. BROWN[3];
[1]Dept Physiol., Univ. Illinois, URBANA, IL; [2]Mol. & Integrative Physiol., [3]Biophysics and Computat. Biol., Univ. of Illinois, Urbana, IL

Abstract: Predator-prey interactions where predators learn to distinguish the sensory cues of valuable versus well-defended prey provide clear examples of cost-benefit decision in foraging. The cognitive adaptations for such distinctions both lend flexibility to the generalist's foraging strategy and add complexity to the animal's behavioral economy. The predatory sea-slug *Pleurobranchaea* is one such simple generalist which can rapidly learn with experience to selectively avoid the odor of dangerous prey (Noboa and Gillette, 2012). Notable exceptions are animals with extremely high or low feeding thresholds that either ignore the dangerous prey or completely consume it, respectively.

What are the computations of this foraging strategy and how are they mediated in the nervous system? We elucidated neuronal circuitry mediating appetitive state in terms of integrating sensation, motivation and memory, and we showed how it controls a neuronal switch for approach-avoidance decision (Hirayama and Gillette, 2013). A neural model for cost-benefit decision was derived from these relations. Here we test the model in computational simulation. In it, sensory modalities and learned associations are integrated in incentivizing and deterrence network circuits that feed separately to a leaky integrator embodying satiation and summing inputs to express appetitive state, and to premotor turn circuitry organized by default for avoidance responses. Reciprocal feed-forward interactions between appetitive state and turning motor output produce a diversity of behaviors, reproducing well the real animal's responses based on sensory modality, learning and motivational state.

Successful reproduction of adaptive cost-benefit decision relations in a simple forager provides an arena for testing effects of varying behavioral and ecological factors on reproductive fitness and environmental change. It also suggests a foundation for the directed development of more complex cognitive and social abilities in computational entities, paralleling their evolutionary acquisition in real animals.

Disclosures: R. Gillette: None. **K. Tian:** None. **N. Ryckman:** None. **J. Brown:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.21/JJJ68

Topic: F.02. Animal Cognition and Behavior

Support: DFG Grant STU544/1-1

Title: Assessing the multidimensional integration of reward value in the pigeon

Authors: *N. KASTIES, O. GUNTURKUN, M. STUTTGEN;
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Abstract: Decision making - choosing a single behavioral option to the exclusion of all others - is a fundamental process underlying all behavior. Naturally, the range of available options is not uniform but rather spans across a vast range of dimensions. Behavioral choice thus involves deciding between options that are in few ways alike and difficult to compare to each other. Current theory holds that this process involves assigning a subjective, unidimensional value to each available option. These values can then be compared easily with the best-scoring option being subsequently executed.
Neuronal correlates of subjective value have been shown in several species, among them primates and rodents. More recent studies have investigated how neuronal activity integrates value across multiple dimensions such as reward probability, effort or delay. Here, we introduce a simple sign-tracking task for pigeons in which discrete stimuli predict a food reward varying along three dimensions: reward amount, time to reward and reward probability. We find that both the probability of responding to a given stimulus as well as the rate of responding monotonically increase with reward value along each dimension: More specifically, stimuli indicating large rewards elicit higher response frequencies than those indicating small rewards; stimuli indicating sooner rewards are likewise favored over those predicting later rewards, and likewise for high vs. low reward probability. This ordinal relation holds true across stimuli differing along more than one reward dimension. We thus identify response frequency to be a reliable behavioral indicator of the animal's reward expectancy. We have begun to record single-neuron spiking activity in the nidopallium caudolaterale (NCL) during this task. The NCL presents an analog of the mammalian prefrontal cortex (PFC), a structure in which reward-related neuronal activity has been repeatedly demonstrated. This experiment aims to identify a neuronal representation of multidimensionally integrated value in the pigeon NCL.

Disclosures: N. Kasties: None. **O. Gunturkun:** None. **M. Stuttgen:** None.

Poster

575. **Decision Making: Behavioral and Pharmacological Studies**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 575.22/JJJ69

Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01MH095894

DOD W81XWH-11-1-0584

FRSQ 25559

Title: Inhaling oxytocin increases contagious yawning in rhesus macaques

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Abstract: Watching another individual yawn induces yawning in humans and other highly social animals such as dogs, macaques and chimpanzees. Such contagious yawning is thought to relate to group coordination or empathy. It has also been shown that individuals with autism fail to yawn in response to seeing others yawn, further suggesting a relationship between yawning contagion and processes such as empathy and social attention. Despite its potential role in social behavior, the neural basis of social yawning remains mysterious. Notably, oxytocin (OT) is known to favor pro-social behaviors, yet its effect on contagious yawning is unknown. Here, we used a behavioral paradigm designed to identify contagious yawning in rhesus macaques during live social interactions and its modulation by exogenous OT. In our experiment, a subject male monkey (M1) is paired for social confrontations of 5 minutes duration with all other male monkeys (M2) from a group of eight. The monkeys sit in primate chairs and face each other, but are otherwise free to behave, and all interactions are recorded on video. Video records are then coded by 2 condition-blind experimenters, using the Tinbergen software suite (Geoff K. Adams) to identify pro-social behaviors, threat displays and yawns. On one half of the sessions, one subject monkey (M1) is given intranasal OT using a nebulizer before the experiment, whereas on the other half he is given intranasal saline via the same method. We use granger causality analysis to determine whether the yawns of M2 are followed by yawns in M1. We found that some monkeys in the control condition show contagious yawning while others do not, similar to individual variations in yawning contagion in humans (F-statistics of granger causality for 3

subject monkeys: 0.03, 4.6, 3.9; significant causality when F > 3.3-3.5). More importantly, we found that OT increases yawning contagion in all subjects tested, including those who did not show yawning contagion in the saline condition (F-statistics, oxytocin condition: 5.4, 5.2, 4.6). This effect was not accompanied by an increase in the frequency of yawning in subject monkeys (5.36 ± 0.51 yawns per five minutes on oxytocin days vs 4.87 ± 0.53 on saline days; $p > 0.05$). These results show that exogenous OT increases yawning contagion in rhesus macaques. This novel experimental paradigm will allow us to understand the neurophysiology and pharmacology of contagious yawning. We will also use this paradigm to identify possible links between yawning and other social behaviors such as threat displays and dominance.

Disclosures: D.L. Xie: None. **J. Gariepy:** None. **E. Du:** None. **M.L. Platt:** None.

576**Poster**

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.01/JJJ70

Topic: F.02. Animal Cognition and Behavior

Support: European Research Council

Research Council of Norway

James S. McDonnell Foundation

Human Frontier Science Program

Title: Activation of granule cells increases neurogenesis in the adult dentate gyrus

Authors: *S. BLANKVOORT[1], M. UEMURA[1], A. TASHIRO[1,2,3];
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Abstract: In the dentate gyrus, new neurons continue to be generated to modify the existing neuronal circuits. The amount of newly-generated neurons is regulated by a variety of factors including animal's experience and neural activity in the dentate gyrus. However, it is not well understood whether there is any functional relevance to where the birth of neurons occurs within the dentate gyrus. Previously we reported that spatially-modulated activity, so called place-cell activity, in the dentate gyrus was found in the proximity of immature neurons (Uemura et al.,

Soc. Neurosci. Abstr. 2011). From this finding, we hypothesized that adult neurogenesis is locally induced by activation of granule cells, a cell type in the dentate gyrus believed to show place cell activity. In order to test this, we experimentally activated granule cells by optogenetic stimulation and examined its effect on adult neurogenesis. First, we established transgenic mice in which ~10% of the granule cells express channelrhodopsin-2 (ChR2). To induce specific activation of granule cells, we delivered light (wave length: 473nm) to the dentate gyrus by connecting an implanted fiber connector to a laser source. 90 minutes after light stimulation (1800x10ms pulses at 10Hz, 6-30mW at the fiber tip) we detected the expression of activity-dependent protein FOS in over 90% of the granule cells, indicating that the light stimulation activated granule cells. Subsequently, we investigated an effect of the light-induced activation on proliferation. For this purpose, the granule cells were stimulated, with the same protocol of light delivery and, after three days, the mice were injected with a proliferation marker BrdU. One day after the BrdU injection, we found an increase in proliferation (3011+/-271 BrdU+ cells/mm3 in ChR2+ mice (n=8), 1547+/-153 BrdU+ cells/mm3 in ChR2- mice (n=8), data given in mean+/-s.e.m., $p<0.001$ in t-test). Further, we found an increase in DCX+ cells/mm3 (4721+/-480 DCX+ cells/mm3 in ChR2- mice (n=8), 9522 +/-856 DCX+ cells/mm3 in ChR2+ mice (n=8), $p<0.001$ in t-test), indicating an increase in neurogenesis. The result demonstrates that activation of granule cells facilitates cell proliferation and neurogenesis in the dentate gyrus, supporting the possibility that certain patterns of neuronal activity such as place-cell activity may locally enhance neurogenesis, by creating a niche for fostering the recruitment of new neurons. The local regulation of neurogenesis by behaviorally-driven neuronal activity may conduct strategic recruitment of newly-generated neurons into behaviorally-relevant neuronal circuits.

Disclosures: S. Blankvoort: None. **M. Uemura:** None. **A. Tashiro:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.02/KKK1

Topic: F.02. Animal Cognition and Behavior

Support: European Research Council

James S. McDonnell Foundation

Human Frontier Science Programme

Title: Role of immature neurons in the adult dentate gyrus for spatial memory processing after initial acquisition

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Abstract: Neurogenesis persists in few regions of the adult mammalian brain including the dentate gyrus. Studies employing various ablation methods have shown that adult born neurons contribute to hippocampus-dependent forms of memory. Memory involves multiple steps starting from initial acquisition, being maintained and finally recalled. Recent studies point towards the involvement of relatively mature adult-born neurons (> 4 week old) in memory process after initial acquisition. Although it was claimed that younger immature neurons do not contribute to the memory process, the issue has not been thoroughly investigated. In this work, we focus on the role of younger cohorts of immature neurons in memory process after initial acquisition.

For this purpose, we constructed a lentiviral vector expressing diphtheria toxin receptor (DTR) and green fluorescent protein (GFP) under the control of 3,5kb promoter sequence from human doublecortin (DCX) gene, which is known to be expressed in late neuronal progenitor cells and immature neurons. The expression of DTR renders infected cells in mice vulnerable to the otherwise harmless diphtheria toxin (DT), which interferes with protein synthesis and leads to cell death. By examining the distribution of GFP expression, we found that, with a single stereotaxic injection into the dentate gyrus of adult mice, the viral vector transduced into about 40% of DCX+ cells in the dentate gyrus. A systemic DT injection into these animals reduced the number of DCX+ cells by 35-45% and that of GFP+ cells to virtually none. We found that immature neurons around two weeks old, but not ones older than four weeks, were ablated by the manipulation.

Using this manipulation, we tested spatial memory retention in a water maze task. Two groups of nine mice each underwent a water maze paradigm consisting of 24 trials in a single day. Immediately after the completion of the training, the mice were given a systemic injection of DT to ablate immature neurons. In a probe trial performed at one week after the training/DT injection, a significant reduction in time spent near the platform position was observed in the manipulated group compared with controls ($p < 0,031$, two-tailed t-test). Our results support that immature neurons aged around 2 weeks old at the time of memory formation, contribute to long-term memory processing after initial acquisition.

Disclosures: A. Luchetti: None. **M. Uemura:** None. **F. Oschmann:** None. **L. Čulig:** None. **A. Tashiro:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.03/KKK2

Topic: F.02. Animal Cognition and Behavior

Support: Research council of Norway

James S. McDonnell foundation

Fuge Midt Norge

European Research Council

Title: NMDA receptor mediates learning-induced circuit formation associated with adult neurogenesis in the dentate gyrus

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Abstract: Dentate gyrus is one of the regions in mammalian brain where new neurons are generated throughout life. Many newly-born neurons die during initial weeks after neuronal birth, while the survived ones are functionally integrated into neuronal circuitry contributing to hippocampal functions. Previously, we reported that NMDA-type glutamate receptor (NMDAR) is involved in the survival (Tashiro *et al., Nature* 442:929-933, 2006) and dendritic growth/maintenace (Nair and Tashiro, *Soc Neurosci. Abst*., 2011) of immature new neurons, which are also known to be affected by hippocampus-dependent memory formation. Here we examined whether NMDAR mediates the learning-induced changes in survival and dendritic morphogenesis of adult-born neurons. For this purpose, we injected a retroviral vector co-expressing Cre recombinase and GFP to the dentate gyrus of floxed-NR1 mice, thereby knocking out the NR1 gene, encoding an NMDAR subunit required for functional receptors, and visualizing dendritic morphology of the NR1 knockout (KO) neurons. Effects of spatial learning on the morphology and survival of new neurons were investigated by subjecting the virus-injected mice to a ***delayed-match-to-place*** version of water maze task at different periods after the injection. Four-day trainings during 10-13 or 15-18, but not 8-11, days after the injection significantly increased dendritic complexity and spine density measured in 21 days old wildtype neurons. Thus, the effects are dependent on the age of immature neurons during spatial learning. We found none of the training paradigms caused the effects in NR1KO neurons, suggesting the involvement of NMDAR. Spatial learning during 10-13 days after the injection significantly

increased the survival of wildtype new neurons (learners; 1302 ± 197 neurons/mm^3, swimmer control; 851 ± 140 neurons/mm^3, $p < 0.006$, group x genotype in repeated measures ANONA, $p<0.05$ in post hoc LSD test), which was not found in NR1KO new neurons. Thus, the results indicate that NMDAR is involved in learning-dependent regulations of the survival and dendritic development of new neurons, which are critical steps to determine new circuits formed by the new neurons. These two circuit forming events seem to be tightly coupled as they have three common features, 1) being mediated by NMDAR, 2) an overlapping maturation stage where they occur, and 3) an overlapping maturation stage where spatial learning affects them, suggesting a mechanistic relationship between the two phenomena. These learning-dependent regulations of circuit formation mediated by adult-born neurons may contribute to information storage in the adult dentate gyrus.

Disclosures: R.R. Nair: None. **A. Luchetti:** None. **I. Åmellem:** None. **A. Tashiro:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.04/KKK3

Topic: F.02. Animal Cognition and Behavior

Support: St.Olavs hospital-NTNU Kontaktutvalget

FUGE Midt-Norge

Research Council of Norway

Title: Chronic fluoxetine treatment increases the dendritic complexity of immature granule cells in the adult dentate gyrus

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Abstract: Adult neurogenesis in the dentate gyrus has been implicated in the therapeutic action of monoaminergic antidepressant drugs, such as fluoxetine. It has been shown that chronic antidepressant treatment requires intact neurogenesis to exert the behavioural effects of monoaminergic antidepressants. A previous study showed that chronic fluoxetine treatment

increases the dendritic complexity of immature neurons. This morphological effect is observed only after chronic, but not acute, treatment as is the case for therapeutic effects in patients, suggesting that the increased dendritic arborization of newborn neurons may be involved in the therapeutic effects of antidepressants.
To characterize the effect of chronic fluoxetine treatment on the morphology of new granule cells at different time points after neuronal birth, a retroviral vector expressing GFP was injected into the dentate gyrus of adult C57 mice. The injection was followed by daily injections of fluoxetine (18 mg/kg body weight) for either two, three, or four weeks until the day of euthanasia. Two morphological criteria were measured to analyse the dendritic arborization of the new neurons, the total dendritic length and the number of branch points.
We found that two-week chronic treatment with fluoxetine increases dendritic complexity of two week-old new neurons. This effect is transient during early maturation because we found that the difference was no longer present in three or four week-old neurons after three or four week chronic treatment. Next, to separate the effect of treatment duration from the age of the neurons, chronic treatment with fluoxetine started two weeks before the injection of the retroviral vector. Fluoxetine was applied for two additional weeks until mice were euthanized for analysis. We found dendritic arborization of the two week-old neurons in mice with four-week treatment was more extensive compared to that in vehicle-treated mice. These results suggest that the morphological effects seem to persist after two weeks of chronic treatment at the level of the whole dentate gyrus, although the morphological effect is restricted to early maturation stage and transient at the level of individual neurons. As a population of immature neurons pass the early maturation stage, younger populations would enter the stage and start showing extensive dendritic arborisation. The observed increase in dendritic arborization may be a result of accelerated maturation. This developmental stage of adult born neurons might be critical for their involvement in therapeutic effects of antidepressants.

Disclosures: I. Aamellem: None. **A. Tashiro:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.05/KKK4

Topic: F.02. Animal Cognition and Behavior

Support: European Research Council

European Comission, Marie Curie IEF Grant 252320

St.Olavs hospital-NTNU Kontaktutvalget

James S. McDonnell Foundation

IBRO Research Grant

Fondation pour la Recherche Médicale

Fondation Bettencourt-Schueller

Title: Chronic antidepressant treatment enhances the capacity for context discrimination in the hippocampus

Authors: *A. SURGET[1], Y. IBARGUEN-VARGAS[1], M. H. BLYSTAD[1], A. TASHIRO[1,2,3]; [1]Kavli Inst. For Systems Neurosci., Norwegian Univ. of Sci. and Technol. and St. Olavs Hosp., Trondheim, Norway; [2]Warwick-NTU Neurosci. programme, Sch. of Biol. Sci., Nanyang Technological Univ., Singapore, Singapore; [3]Warwick-NTU Neurosci. programme, Sch. of Life Sci., Univ. of Warwick, Coventry, United Kingdom

Abstract: Although the hippocampus has been shown to be critically involved in antidepressant (AD) effects, the underlying mechanisms are poorly understood. The effects of ADs on the hippocampus have been found by molecular, cellular and behavioral analyses. However, much less is known about the effects on neuronal activity in behaving animals, which are essential for understanding how ADs alter brain functions to exert its therapeutic effects. Here we examined how AD treatments affect neuronal activity in the hippocampus, using place-cell activity in the CA3 area as a model.

For this purpose, male Long Evans rats were implanted with 14-tetrode arrays for multiple unit recording. Following 4-week fluoxetine AD treatment (10mg/kg/day) and training in a familiar environment (an enclosure in a room), recordings were conducted under conditions in which (1) the distal but not proximal cues were changed (the familiar enclosure in a new room) and then (2) the proximal but not distal cues varied (enclosures with walls with familiar versus novel colors in the familiar room). Spatially-modulated activity was assessed from putative CA3 pyramidal cells.

In condition (1), we observed changes in spatial firing patterns and peak firing rates between two rooms (i.e. global remapping) as shown in previous studies. We did not find a significant difference between vehicle- and fluoxetine-treated rats.

In condition (2), while the spatial firing patterns remained unchanged between the different enclosures, the peak firing rates significantly varied between them (i.e. rate remapping), as expected from previous studies. The effect was found to be significantly greater following AD treatment. Moreover, the firing rate changes between the first and last sessions in the familiar enclosure were found to be as high as that between enclosures with different colors in vehicle-treated rats, indicating that an experience in a slightly-modified context may interfere with the representation of the familiar context. This effect was suppressed in fluoxetine-treated rats.

The results indicate that chronic fluoxetine treatment may affect information processing in the CA3 area, by enhancing a difference in the neuronal response to subtle contextual changes and by reducing interferences between representations. We propose that such enhancement in disambiguating equivocal contextual information in the hippocampus may result in providing downstream brain regions with a better discriminating capacity, enabling behavioral response to be appropriately set with the context. This effect may be essential for patients to cope with stressful situations and to alleviate depression.

Disclosures: A. Surget: None. **Y. Ibarguen-Vargas:** None. **M.H. Blystad:** None. **A. Tashiro:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.06/KKK5

Topic: F.02. Animal Cognition and Behavior

Support: Marie Curie fellowship (EU FP7)

the European Research Council

the James S. McDonnel Foundation

Title: Optogenetic identification of granule cell activity in the dentate gyrus of behaving mice

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Abstract: The extracellular unit recording technique with tetrodes (a bundle of four electrodes) is a widely used method for recording neuronal activity in behaving animals. Simultaneously recorded spikes from different neurons are separated by spike sorting based on the amplitudes and shapes of the spikes as they appear differently on each of the four channels. While the technique can identify neuronal types based on their firing properties, its limitation is the inability to determine which anatomically defined cell is the source of the spikes. In the dentate gyrus (DG), unit recording technique detected a few electrophysiologically-defined cell types which are proposed by different studies to correspond to different anatomically-defined cell

types (Neunuebel, 2012 (J Neuro); Leutgeb et al, 2007 (Science); Jung and McNaughton, 1993 (Hippocampus)). Thus, it is unknown how granule cells (GCs), the major anatomically-defined type of output neurons in the dentate gyrus, are activated even though we have information on distinct firing patterns recorded in the dentate gyrus. In this study, we aim to implement a technical approach overcoming this limitation (Lima, 2009; Zhang et al., 2013) and to record from identified GCs in the DG of behaving mice. We expressed the Channelrhodopsin-2 (Chr2) gene encoding a light-sensitive cation channel in GCs. In this way, these neurons are tagged so that they respond to light stimulation by generating spikes. Light-induced firing can then be used to identify the activity of targeted neurons in behaving animals. We have been working with transgenic animals that sparsely express Chr2 in DG GCs (POMC-Cre x B6;129S-Gt(ROSA)26Sortm32(CAG-COP4*H134R/EYFP)Hze/J). We used a chronically implanted micro drive with four independently moveable tetrodes and a separately implanted optic fiber. Light pulses (472nm) of 3ms induced spikes with short latency (5-10ms) in the transgenic animals but not in Chr2 negative controls. This short latency suggests that light-induced activation is not synaptic but direct. Variability in spiking latency is substantial in most cases and spiking probability low. With increasing light intensity the variability in spiking latency is reduced and the probability increases. Taken together these results suggest we can indeed directly activate GCs that express Chr2 and record the light-induced activity. This result represents a major step towards our goal by showing that this is a promising approach to identify GCs in behaving mice. We are optimizing the tetrode and optic fiber configuration to target them more precisely to the same area of the DG.

Disclosures: N.Z. Borgesius: None. **A. Tashiro:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.07/KKK6

Topic: F.02. Animal Cognition and Behavior

Support: Research council of Norway

European Research Council

Grants-in-Aid for Science Research on Innovative Areas, "Mesoscopic Neurocircuitry"

Funding Program for Next Generation World-Leading Researchers

Title: Role of synaptic plasticity in controlling information flow in the hippocampus during novelty

Authors: *T. KITANISHI[1], S. UJITA[2], M. FALLAHNEZHAD[1,3,4], N. KITANISHI[1], Y. IKEGAYA[2,5], A. TASHIRO[1,3,4];
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Abstract: Synaptic plasticity is believed to be a general cellular mechanism by which neurons acquire and store information. The learning experience induces dynamic rearrangement of neuronal firing patterns in the brain, which may reflect the acquisition of learned information in neuronal network. Although the changes in neuronal firing are often assumed to be mediated by synaptic plasticity, direct evidence for the causal relationship is scarce. Here we devised a novel virus-mediated approach to perform unit recording from genetically manipulated neurons in the intact brain environment and examined a role of synaptic plasticity in novelty-induced changes of neuronal activity, using place cells in the hippocampal CA1 area as a model. We show that a form of synaptic plasticity dependent on the GluR1 subunit of AMPA receptor mediates rapid formation of spatial firing patterns in place cells upon exposure to novel environments. Further we show that, during novel experience, GluR1-dependent synaptic plasticity determines how temporal firing patterns of CA1 neurons are modulated by slow gamma oscillations, which is thought to originate from the CA3 region. These results suggest that GluR1-dependent synaptic plasticity at CA3-CA1 synapses is a cellular mechanism by which neurons acquire spatial and temporal firing patterns during novel experiences. We propose that GluR1-dependent synaptic plasticity determines information flow between subregions in the hippocampal-entorhinal system, which may be a general role of synaptic plasticity at the circuit level.

Disclosures: T. Kitanishi: None. **S. Ujita:** None. **M. Fallahnezhad:** None. **N. Kitanishi:** None. **Y. Ikegaya:** None. **A. Tashiro:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.08/KKK7

Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant 5R01MH08368604

NINDS grant 5RC1NS06814802

Helen Hay Whitney Fellowship

Title: Using virtual reality to study hippocampal remapping in rats during 2D navigation

Authors: *D. ARONOV, D. W. TANK;
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Abstract: Hippocampal remapping is a process by which hippocampal place cells change their spatial representation whenever the animal's environment or the behavioral context changes. This process is thought to reflect the expression of distinct spatial memories within the hippocampal network. To understand the neural mechanisms of remapping, it is beneficial to study the animal in different contexts that can be switched or modified in a temporally precise way.

Because virtual reality (VR) is a technique that allows such manipulations, we designed and implemented a VR system for rats. In this system, animals navigate by walking on a spherical treadmill and viewing a projected image of a virtual environment on a full-surround screen. Instead of being head-fixed, rats in our setup are body-tethered to a commutator (Hölscher et. al. 2005), which allows them to freely rotate and walk in any direction on the treadmill. We found that, in this setup, rats perform a wide range of natural 2D behaviors, such as walking in direct paths to targets, making abrupt stops and sharp turns, and avoiding walls of the virtual environment.

To evaluate our system as a tool for studying remapping, we designed two virtual environments that differed in shape (square, 1x1m or circle, 1.4m diameter) and the visual cues. Animals were trained on separate 30-min sessions in the two environments. Each environment was divided into 7-16 invisible equal-area zones, and one of the zones was randomly designated as the "reward zone." When a rat successfully entered the reward zone, a reward was delivered, and a different reward zone was selected. Animals successfully learned to obtain >100 rewards per session and produced full coverage of each environment.

We recorded CA1 neurons using tetrodes and a multiplexing extracellular recording system built into the VR setup. We observed 2D place fields that were similar in size and shape to those reported in real-world environments. We also observed global hippocampal remapping across the two environments: some cells exhibited a place field in only the square or the circular environment, whereas other cells exhibited fields in both, but at different relative locations. In a separate group of rats, we recorded neurons in the superficial layers of the medial entorhinal cortex and observed grid cells with hexagonally-arranged firing fields, similar to those reported in real-world environments.

The virtual-reality apparatus that allows well-controlled changes in environment, combined with neural recordings of place cells and grid cells, creates new opportunities to study the neural mechanisms by which different contexts are represented in the hippocampal network.

Disclosures: D. Aronov: None. **D.W. Tank:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.09/KKK8

Topic: F.02. Animal Cognition and Behavior

Title: Selective activation of M1 and M4 muscarinic receptors and object recognition memory performance in rats

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Abstract: Acetylcholine (ACh) signaling through muscarinic receptors (mAChRs) can improve memory performance on some tasks, but peripheral side effects often accompany pan-mAChR activation. Drug therapies that selectively activate M_1 or M_4 muscarinic receptors at allosteric binding sites may be able to improve memory performance without causing the peripheral side effects that are primarily associated with the M_2 or M_3 mAChR subtypes. The ability of drugs selective for M_1 or M_4 receptors to improve memory performance was tested by giving long-Evans rats subcutaneous (*s.c.*) injections of three different doses of the M_1 allosteric agonist VU0364572, the M_1 positive allosteric modulator (PAM) BQCA, or the M_4 PAM VU0152100 before performing an object recognition memory task. Each rat also completed three different sessions with 0.9% saline *s.c.* injections to establish baseline performance. The lowest dose (3.0 mg/kg) of VU0152100 markedly improved memory performance of rats who performed poorly at baseline without altering the general tendency of rats to explore novel objects. These results offer support that drug therapies that selectively target M_4 receptors show promise to improve memory performance of individuals with impaired memory, such as patients with Alzheimer's disease or schizophrenia. Future studies will use *in vivo* electrophysiology to examine if and how these muscarinic activators affect hippocampal function during object recognition memory performance.

Disclosures: C.R. Galloway: None. **E.P. Lebois:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vanderbuilt University. **N.A. Hernandez:** None. **S.L. Shagarabi:** None. **J.R. Manns:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.10/KKK9

Topic: F.02. Animal Cognition and Behavior

Title: Effects of selective activation of M1 muscarinic receptors on hippocampal oscillations and spatial representations in rats

Authors: *E. P. LEBOIS[1], M. J. HAMM[2], A. I. LEVEY[1], J. R. MANNS[2];
[1]Neurol., [2]Psychology, Emory Univ., Atlanta, GA

Abstract: Muscarinic acetylcholine receptors (mAChRs) are a family of five cholinergic G-protein coupled receptors expressed throughout the brain and periphery. The M_1 receptor is heavily expressed in several forebrain regions, including those important for memory such as the hippocampus. Pharmacological work has shown that selective M_1 activation improves spatial memory, and *in vitro* studies with M_1 knockout mice have indicated that M_1 activation suppresses transmission at CA3-CA1 synapses. However, the effects of selective M_1 activation on spatial representations and CA3-CA1 functional connectivity in awake, behaving animals remains unclear. The present work used *in vivo* tetrode recording from dorsal CA3 and CA1 subregions to obtain spiking and local field potential data in adult rats during open field exploration. During exploration, the walls of the recording enclosure were incrementally reshaped to assess the influence of spatial changes on hippocampal place fields. The M_1 agonist, VU0364572, and the M_1 positive allosteric modulator, BQCA, were used to examine the impact of selective M_1 activation on hippocampal place fields and CA3-CA1 synchrony. Consistent with prior pharmacological and *in vitro* findings, the present results indicated that M_1 activation can decrease measures of CA3-CA1 functional connectivity yet benefit spatial representations. Specifically, selective M_1 activation decreased CA3-CA1 spike-field synchrony in the theta range. However, selective M_1 activation also increased responsiveness of hippocampal place fields to changes in the shape of the recording enclosure. CA1 receives input from both CA3 and entorhinal cortex (EC), and EC-CA1 synaptic transmission is thought to be modulated by nicotinic acetylcholine receptors more so than muscarinic receptors. Thus, one possible interpretation of the present results is that less coherent CA3-CA1 synaptic transmission resulting from M_1 activation might act to prioritize information coming from entorhinal cortex about current spatial stimuli. As the current work involved systemic administration of the drugs, further research will be needed to determine the extent to which M_1 receptor activation specifically in the hippocampus mediated the observed effects. Nevertheless, the results provide insight as to how muscarinic receptors mediate the balance of network dynamics in the

hippocampus and highlight potential therapeutic targets for treating age-related cognitive decline and Alzheimer's Disease.

Disclosures: E.P. Lebois: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vanderbilt University. **M.J. Hamm:** None. **A.I. Levey:** None. **J.R. Manns:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.11/KKK10

Topic: F.02. Animal Cognition and Behavior

Title: Temporal context and recognition memory in rats

Authors: *J. B. TRIMPER[1], C. R. GALLOWAY[1], N. A. HERNANDEZ[2], S. L. SHAGARABI[1], P. B. SEDERBERG[3], J. R. MANNS[1];
[1]Psychology, Emory Univ., Atlanta, GA; [2]George State Univ., Atlanta, GA; [3]The Ohio State Univ., Columbus, OH

Abstract: Memories for events are influenced by the spatial context in which the events occur. These event memories can also be influenced by memories for preceding events, which can be considered a form of temporal context. The present experiment used an object recognition memory paradigm in rats to ask how memory for an object encounter might be influenced by prior object encounters. Rats (n=10) encountered separately three junk objects on each of two laps around a circular track. Performance was evaluated by measuring the amount of time rats voluntarily inspected objects. A key question centered on the extent to which exploration of the third object on the second lap was influenced by whether the first two objects presented were novel or repeated. The results indicated that, when the third object was novel, rats explored it more if the preceding two objects had been encountered on the previous lap as compared to if the preceding two objects were also novel. One interpretation for this pattern of results is that encountering a repeated initial pair of objects elicited the memory of the third object from the previous lap. By this view, the longer exploration time resulted from a mismatch between the current object and the memory of the third object from the previous lap. If true, the results would highlight a tractable means to operationalize and test the influence of temporal context on object recognition memory in rats and would enable future studies to investigate the role of the hippocampal memory system in this aspect of memory.

Disclosures: J.B. Trimper: None. **C.R. Galloway:** None. **N.A. Hernandez:** None. **S.L. Shagarabi:** None. **P.B. Sederberg:** None. **J.R. Manns:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.12/KKK11

Topic: F.02. Animal Cognition and Behavior

Support: NIMH F30MH095491

Emory University Research Committee

Title: Electrical stimulation of the amygdala elicits Gamma coherence between CA1 and CA3 in the hippocampus

Authors: *D. I. BASS[1], Z. NIZAM[2], A. WANG[2], M. JOSEPH[3];
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Abstract: Emotionally arousing events can lead to activation of the basolateral complex of the amygdala (BLA), often enhancing consolidation of memory for these events. Research in rats has shown that direct¬ly manipulating the BLA can enhance memory for non-arousing, emotionally-neutral events, as well. For example, we previously reported that brief electrical stimulation of the BLA in rats enhanced object recognition memory in a stimulus-specific manner (Bass, D.I., Partain, K.N., and Manns, J.R., 2012, Behav Neurosci, 126:204-8). In particular, only objects whose initial exploration was immediately followed by electrical stimulation of the BLA were remembered one day later. One possible interpretation of those results was that stimulation of the BLA benefitted memory by modulating activity in the hippocampus. Indeed, a single pulse to the BLA can elicit an evoked potential in regions of the hippocampus that receive direct projections. In the present study, rats performed a novel object recognition memory task while local field potentials and single-unit activity were recorded in the hippocampus. As in our original study, the initial exploration of some of the objects was immediately followed by brief electrical stimulation to the BLA. Preliminary analyses indicate that electrical stimulation of the BLA did not modulate the gross firing rate of hippocampal neurons. However, BLA stimulation corresponded to increased coherence in the low gamma band (30-40Hz) between CA1 and CA3 ipsilateral to the side of stimulation. This finding is consistent with previous studies that have found gamma synchrony in the hippocampus is associated with improved performance on hippocampus-dependent memory tasks. Further

experiments will be needed to determine whether CA1-CA3 gamma coherence is necessary for the improved memory consolidation associated with BLA activation.

Disclosures: D.I. Bass: None. **Z. Nizam:** None. **A. Wang:** None. **M. Joseph:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.13/KKK12

Topic: F.02. Animal Cognition and Behavior

Support: Human Frontiers Science Grant RGP0039/2010

Title: Deliberative decision making: Is the hippocampus necessary for vicarious trial and error behaviour and performance in a spatial delay discounting task?

Authors: *D. BETT[1], L. H. MURDOCH[1], S. ANASTASSIOU[1], E. R. WOOD[1], P. A. DUDCHENKO[1,2];
[1]CCNS, Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Dept. of Psychology, Univ. of Stirling, Stirling, United Kingdom

Abstract: Vicarious trial and error (VTE) is a side to side movement of the head displayed when a rodent faces a choice-point on a maze. This behavior has been linked to spatial learning and the hippocampus (HPC) (Amsel, 1993) and has been associated with forward sweeps of CA3 place cells at a choice point (Johnson and Redish, 2007). Previous work from our group showed that HPC damage does not abolish VTE behaviour on a serial reversal double Y-maze task (Bett et al., 2012). However, in control rats (but not rats with HPC damage), VTE was higher during exploration trials compared to trials once the food was located. In the current experiment, we investigated hippocampal involvement in VTE and performance on a spatial delay discounting task.
Rats were trained on a spatial delay discounting task (Papale et al., 2012) prior to receiving ibotenic acid lesions of the HPC or sham lesions. Rats were trained to collect food rewards at the end of each arm on a continuous T-maze; a high reward (HR; 3 food pellets) was available on one arm after an adjustable delay and a low reward (LR; 1 food pellet) was available on the opposite arm after a fixed delay of 1 s. After choosing the HR the delay was increased by 1 s, whereas after choosing the LR arm the delay was decreased by 1 s; alternation resulted in a no change in delay. The initial delay value was changed daily between 1 and 30 s. Rats typically biased their choices to one side or the other, thereby changing the adjustable delay value up or down until an indifference point (IP) was reached between the HR and LR. VTE behaviour was

significantly higher during trials in which the rats repeated their choice of arm compared to trials in which the rats alternated their choice, suggesting more VTE during the titration of the HR value.
Following surgery, there was no difference between the two groups in IP or VTE across all initial delays. However, a mild effect of hippocampus lesion was found. The mean IPs of the lesion group was lower than the control group at short initial delays. VTEs were also lower at short initial delays for the hippocampus lesion group. This suggests that the HPC may modulate VTE behaviour and also delay discounting but only when the initial delay is low.
The rats were then tested on a spatial delay discounting task on a simple T-maze. In this task, rats received either a HR or LR at the end of each arm. When a 10 s (non-adjusting) delay was introduced before receiving the HR, rats with hippocampus damage were as likely as control rats to choose the HR arm, contrary to previous findings (McHugh et al., 2006). This may be an effect of prolonged training in the adjusting-delay spatial delay discounting task.

Disclosures: D. Bett: None. **L.H. Murdoch:** None. **S. Anastassiou:** None. **E.R. Wood:** None. **P.A. Dudchenko:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.14/KKK13

Topic: F.02. Animal Cognition and Behavior

Support: HFSP

Title: Hippocampal place cells in CA3 encode future destinations on a double Y maze

Authors: E. ALLISON[1], P. A. DUDCHENKO[2], *E. R. WOOD[1];
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Abstract: Place cells in the hippocampus fire in specific locations within an environment. In addition to spatial modulation, place cell activity can also be modulated by relevant features of a task. For instance, in a task in which rats are trained to run along a common path on their way to spatially distinct goal locations, a significant proportion of CA1 place cells with fields in the common path show activity which is modulated by which goal the rat is going to. This differential activity has also been recorded in the entorhinal cortex and in CA3 but it is not known where and how it originates. The purpose of this study was to determine whether prospective place cell activity occurs in CA3 in a hippocampus-dependent serial reversal task on the double Y maze and to compare it with that seen in CA1. We recorded simultaneously from

single cells in CA3 and CA1 in rats trained on a serial reversal task on a double Y maze. Rats were trained to run from a start box through two Y-junctions to one of four goal locations. After 10 trials the reward was moved to a new location, until all the boxes had been rewarded. Previous research has found that 44% of CA1 place cells with fields in the start areas of the maze show trajectory dependent activity in rats trained on the task. The new result that we observed is that a similar proportion of CA3 place cells also show trajectory dependent activity in rats trained on this task. This result confirms previous findings of trajectory dependent activity in CA3 and extends it to a novel task.

Disclosures: E. Allison: None. **E.R. Wood:** None. **P.A. Dudchenko:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.15/KKK14

Topic: F.02. Animal Cognition and Behavior

Support: Human Frontiers Science Program RGP0039/2010

Title: Do place cells encode goals or routes?

Authors: R. M. GRIEVES[1], E. R. WOOD[2], *P. A. DUDCHENKO[1];
[1]Univ. Stirling, Stirling, United Kingdom; [2]Ctr. for Cognitive and Neural Systems, Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: In a previous study, Ainge et al. (2007) found that place cell firing on the initial stem of a double Y-maze predicted which goal box a rat would navigate to. However, the four goals on this apparatus differed not only in their location, but also in the route taken to each. Therefore, it was unclear whether this anticipatory place cell firing reflected the encoding of a specific goal box, or the encoding of a specific route.
To distinguish between these alternatives, we constructed a modified double Y-maze, where two routes lead to the same goal box. If place cell activity encodes goals, then firing on the common stem of the maze should occur regardless of which route the rat takes to the common goal box. Conversely, if place cells encode specific trajectories, anticipatory firing on the common stem of the maze should occur for one route, and not the other.
To date, we have recorded from 113 place cells with place fields in the start box or the initial stem of the maze. Out of 46 cells that fired when the animal ran to the common goal box, 45 (98%) fired when the rat took one route to the common goal box, and not the other. Only one cell has shown similar firing for both routes to the common goal. Therefore, when rats are trained to

use a specific trajectory to reach a goal, anticipatory place cell firing reflects the trajectory being followed rather than (or as well as) the ultimate goal destination. One possibility is that because rats were trained on a route strategy, their place fields reflected the encoding of routes. To test this, we are training a second group of rats where the common goal box is reinforced regardless of the route taken to reach it.

Disclosures: R.M. Grieves: None. **E.R. Wood:** None. **P.A. Dudchenko:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.16/KKK15

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

CFI

Ontario MRI ERA

Sloan Foundation

Title: Saccadic eye movements are phase aligned to a low frequency oscillation in the macaque hippocampus

Authors: *R. MONTEFUSCO-SIEGMUND, T. K. LEONARD, K. L. HOFFMAN;
York Univ., Toronto, ON, Canada

Abstract: Active sensing generates repetitive movements that can modulate or entrain oscillatory activity in the brain. In active vision, saccadic eye movements align intrinsic ~3 Hz oscillations in visual cortex to their optimal phase and lead to faster response times. The effects of active sensing are not limited to primary sensory areas. In rodents, hippocampal theta-band activity (centered around 8 Hz) is modulated by whisking and sniffing, as well as locomotion. In primates, the role of active sensing on hippocampal oscillations is unclear, though the dependence on visual exploration suggests that saccades may be an important rhythmic movement to align to. The effects due to eye movements might be seen in slower frequencies, based on visual cortex modulation (3 Hz) and the slower, less periodic movement of saccades compared to other active sensing movements.
To study the relationship between low frequency oscillations of the hippocampus and visual exploratory behavior, we examined the hippocampal local field potentials (LFPs) aligned to

fixation onset in the macaque during a flicker change blindness visual search task. We found the strongest oscillatory modulation at 1.7 Hz during search behavior, with the strongest, significant phase consistency occurring 70 ms prior to fixation onset, near the trough of the 1.7 Hz oscillation. Shuffling saccade intervals within a trial removed the effect. One likely stimulus-related artifact was the onset times of the flashing images, which occurred at the same frequency as our oscillation of interest. If saccades occurred with an uneven distribution around image onset, and if image onset produced an evoked response, then a spurious phase concentration to eye movements would emerge. To control for such image-evoked effects, we measured phase concentration of LFP locked to fixations that occurred during the 20-second inter-trial interval, when the screen was an unchanging black, in a darkened booth. We found the same modulation as during scene search, suggesting the effect was not a direct reflection of the changing visual stimulus. If the oscillation is present irrespective of task or visual stimulus dynamics, it may reflect an intrinsic process that may pace the physical sampling of the environment, bringing movement and ongoing neural activity into register.

Disclosures: R. Montefusco-Siegmund: None. **T.K. Leonard:** None. **K.L. Hoffman:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.17/KKK16

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

CFI

Ontario MRIERA

Sloan

Title: Cross frequency interactions in macaque hippocampus surrounding sharp wave ripple events

Authors: *J. M. MIKKILA, T. K. LEONARD, R. MONTEFUSO-SIEGMUND, K. L. HOFFMAN;
York Univ., North York, ON, Canada

Abstract: Oscillations in neural populations occur in distinct frequency bands, and these bands may show a higher-order nesting - or cross-frequency coupling - that varies by brain region,

cognitive processing, and performance. One well-described example of cross-frequency coupling is the amplitude of gamma oscillations which are modulated by the phase of theta oscillations in the hippocampus of rodents during spatial exploration [1]. Another canonical hippocampal event known as a sharp-wave ripple (SWR) was shown to be amplitude-modulated by the phase of oscillations in the 20-50 Hz frequency band, in the rodent performing a spatial alternation task [2]. SWRs have also been described in the primate, albeit primarily during quiet wakefulness and sleeping behaviors. We observed SWRs during visual exploration in the macaque, and asked whether they act independently or are coupled with other hippocampal oscillations. During the search task [3], power was concentrated around 10 Hz, 20-35 Hz and >50 Hz, including SWR events. Similar to findings in the rodent, we observed a coupling of ripple amplitude with the phase of a 20-25 Hz oscillation. In contrast to the phase-amplitude coupling results, power was decoupled between the ripple and all non-sharp-wave bands at the time of the ripple event. After the ripple, power in a 20-25 Hz band increased relative to pre and inter-ripple levels, and co-varied with the power of the immediately preceding ripple. These findings reveal a segregation of phase and amplitude effects in cross-frequency interactions, and will be discussed in the context of their possible relevance to behaviour.
[1] Jensen, O. & Colgin, L. L. (2007). Trends Cogn. Sci. 11:267-9.
[2] Carr, M. F., Karlsson, M. P. & Frank, L. M. (2012). Neuron 75:700-713.
[3] Chau, V.L., Murphy, E.F., Rosenbaum, R.S., Ryan, J.D. & Hoffman, K.L. (2011). Front. Behav. Neurosci. 5: doi: 10.3389/fnbeh.2011.00058

Disclosures: J.M. Mikkila: None. **T.K. Leonard:** None. **R. Montefuso-Siegmund:** None. **K.L. Hoffman:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.18/KKK17

Topic: F.02. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute International Student Research Fellowship

Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarships

Searle Scholars Program

Human Frontier Science Program

Kavli Institute at Columbia University

Title: Imaging Ca2+ activity in GABAergic septo-hippocampal projecting axons during awake behaviour

Authors: *P. KAIFOSH, M. LOVETT-BARRON, G. F. TURI, A. LOSONCZY;
Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: Hippocampal interneurons are selectively targeted by subcortical GABAergic projections. To characterize the population dynamics of the GABAergic septo-hippocampal projection in awake behaving animals, we developed a system for 2-photon imaging of axon terminals from this projection in the hippocampus of head-fixed mice walking on a treadmill while engaged with external sensory stimuli. We virally expressed GCaMP.5 in GABAergic septal neurons, and imaged Ca2+ dynamics in their identifiable axonal boutons making putative synaptic contacts with somatic or dendritic profiles of tdTomato-labeled interneurons in hippocampal area CA1. We found subgroups of boutons that behaved coherently during resting, locomotion, and/or sensory stimulation, but were silent under anesthesia. With local application of baclofen through a hole in the imaging window, and by tracing axon segments between boutons, we found that GABA-B receptor-mediated presynaptic inhibition modulates the amplitude, but not the tuning properties, we demonstrated that these highly correlated activitiy patterns resulted from shared presynaptic origin, rather than local neuromodulation of septo-hippocampal bouton responses. We also developed techniques for simultaneous bouton imaging and hippocampal LFP recording, which we applied to investigate the relationship between sensory-evoked bouton activity and hippocampal theta rhythms.

Disclosures: P. Kaifosh: None. **M. Lovett-Barron:** None. **G.F. Turi:** None. **A. Losonczy:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.19/KKK18

Topic: F.02. Animal Cognition and Behavior

Title: Field potential and unit activity from hippocampal regions in urethane anesthetized fruit bats, Carollia perspicillata

Authors: R. ORMAN, M. STEWART, *S. E. FOX;
Dept Physiol & Pharmacol, Downstate Med. Ctr., Brooklyn, NY

Abstract: Several species of bats have received attention as model animals for studies of navigational brain systems in hippocampal and parahippocampal regions. Similarities in spatial firing properties of hippocampal place cells and medial entorhinal grid cells in bats and rats are contrasted with evidence that bats do not exhibit theta frequency activity.
Histological studies of hippocampal formation regions were done to establish stereotaxic coordinates for our forebrain atlas (Scalia, Rasweiler, Scalia, Orman, Stewart, Forebrain atlas of the short tailed fruit bat Carollia perspicillata, Springer, in press). We anesthetized male and female fruit bats with urethane and explored hippocampal regions with field potential and unit recordings. In this initial survey we have recorded theta frequency local field potentials (LFPs) from area CA1.
We conclude that bats may generate hippocampal theta activity, but completion of our ongoing single unit and current source density analyses will be necessary to conclusively demonstrate the hippocampal origin of these field potentials in bats.

Disclosures: R. Orman: None. **M. Stewart:** None. **S.E. Fox:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.20/KKK19

Topic: F.02. Animal Cognition and Behavior

Support: DOD W81XWH-07-2-0092

Title: Hippocampal remapping involves competition between entorhinal inputs

Authors: *J. DICKINSON[1], A. WEIBLE[2], D. ROWLAND[3], C. KENTROS[3];
[1]Inst. of Neurosci., [2]Univ. of Oregon, Eugene, OR; [3]NTNU, Trondheim, Norway

Abstract: Both medial entorhinal cortex (MEC) neurons and their downstream pyramidal targets in the hippocampus have spatial receptive fields. “Grid” cells of the MEC have multiple receptive fields arranged in a hexagonal pattern which largely stay the same in different environments, whereas hippocampal place cells have single, environmentally-specific place fields. This leads to the question of whether, and how, grid cells may create place fields. Exposure to environmental novelty leads to drastic, unpredictable changes in place cell firing patterns (remapping). To investigate how grid cells contribute to place cell remapping, we expressed the hM3Dq DREADD (Designer Receptor Exclusively Activated by Designer Drugs), a modified muscarinic G-protein coupled receptor exclusively activated by clozapine-N-oxide (CNO, an otherwise inert metabolite of the antipsychotic clozapine) in MEC layer II projection

neurons. Since binding of CNO to hM3Dq results in a depolarization lasting for several hours, CNO injection in these mice allows us to investigate the network response to manipulating a random subset of upstream entorhinal inputs against the backdrop of a fully formed and consolidated spatial map. Previously, we showed that increasing MEC LII activity caused some CA1 place fields to remap, while others expanded their firing fields or were unaffected, much like the response to environmental novelty. This "artificial remapping" allows the investigation of how the grid-like inputs to the hippocampal network affect place cells, and help evaluate theoretical models of the putative grid-to-place cell transformation. To address these questions, we analyzed CA1 place fields of the same cells during multiple CNO injections over multiple days. Our data show a range of responses of CA1 neurons to depolarizing a subset of entorhinal inputs. CNO often induced clear remapping to a new field, but we also saw a variety of intermediate responses such as multiple fields, alternation between two place fields, and place field expansions. This suggests that there are instances where the network-level competition does not go to completion, which enables us to effectively identify intermediate states of the network that reveal part of the grid-to-place cell transformation. These results suggest that the inputs to pyramidal neurons compete to determine a single place field in an attractor-like manner.

Disclosures: J. Dickinson: None. **A. Weible:** None. **D. Rowland:** None. **C. Kentros:** None.

Poster

576. **Hippocampus: Subiculum Physiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 576.21/KKK20

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

BMBF

CAPES

FAPERN

SFB 636/B06

Title: Olfactory-driven oscillations in the mouse hippocampus

Authors: *A. B. TORT[1], Y. YANOVSKY[2], M. CIATIPIS[3], A. VYSSOTSKI[4], A. DRAGUHN[2], J. BRANKACK[2];
[1]Neurosci., Brain Institute, UFRN, Natal, Brazil; [2]Inst. for Physiol. and Pathophysiology, [3]Dept.

of Orthopedics, Trauma Surgery and Spinal Cord Injury, Heidelberg Univ., Heidelberg, Germany; [4]Inst. of Neuroinformatics, Univ. of Zürich, Zürich, Switzerland

Abstract: Olfaction is the dominant sense in rodents. Local field potentials (LFP) in the olfactory bulb (OB) exhibit prominent respiration related oscillations. Although the OB sends projections to the entorhinal cortex, little is known about the influence of olfactory inputs on network oscillations in the hippocampus, a region that integrates polymodal information required for memory formation. Here we report a robust 2 to 4 Hz LFP rhythm in the hippocampus of urethane-anesthetized mice that is highly coherent with the OB rhythm and respiratory activity. The amplitude of the hippocampal olfactory rhythm varied across the CA1-dentate gyrus (DG) axis and was maximal at the hilus. In contrast, simultaneous theta (4 to 6 Hz) oscillations were maximal near the hippocampal fissure. Concurrent theta and olfactory oscillations could be further disentangled: (1) atropine abolished theta oscillations but did not alter the olfactory rhythm; (2) bypassing nasal airflow through tracheotomy abolished the olfactory rhythm but not theta oscillations. Extracellularly-recorded hippocampal spiking activity phase-coupled to both rhythms. Intracellular recordings from DG neurons revealed subthreshold membrane potential which oscillated at the same frequency and in anti-phase with the olfactory LFP rhythm. Finally, we found similar 2 to 4 Hz oscillations in the hippocampus of awake mice that also exhibited strong coherence with the OB rhythm. These results demonstrate that nasal airflow leads to hippocampal oscillations phase-locked to respiratory activity, which are likely generated by entorhinal cortex inputs to DG. This olfactory-driven oscillation is clearly different from the hippocampal theta rhythm.

Disclosures: A.B. Tort: None. **Y. Yanovsky:** None. **J. Brankack:** None. **A. Draguhn:** None. **M. Ciatipis:** None. **A. Vyssotski:** None.

577**Poster**

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.01/KKK21

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

CAPES

FAPESC

FAPESP

Title: Involvement of prelimbic cortex cannabinoid type-1 receptors in the disruptive effect of cannabidiol on contextual fear memory reconsolidation

Authors: *C. J. STERN[1], L. GAZARINI[1], M. S. HAMES[1], A. W. ZUARDI[2], R. N. TAKAHASHI[1], L. J. BERTOGLIO[1];
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Abstract: The medial prefrontal cortex (mPFC) has been implicated in fear memory processing. The prelimbic (PL) part of the mPFC is connected with brain regions controlling memory reconsolidation, a process that occurs after the retrieval/reactivation of a consolidated memory and that makes it susceptible to interferences. Several pharmacological agents have been shown to disrupt memory reconsolidation, but the neural substrate underlying this effect remains unclear. The objective of the present study was to investigate the potential role of PL in fear memory reconsolidation. It was also of interest to investigate whether PL is a neural substrate for the disruptive effects of the phytocannabinoid cannabidiol (CBD) on this memory stage. The protocol consisted of familiarization, fear conditioning (0.8 mA, 3 s, inter-shock interval: 30 s), memory reactivation and test A in the context A. A neutral chamber B was used in the non-reactivated protocol and a chamber C was used to the reinstatement protocol. Contextual fear memory was inferred from the percentage of freezing behavior. Immediately after reactivation, male Wistar rats received a bilateral PL infusion of vehicle (VEH) or muscimol (MUS; 4.0 nmol/side), a gamma-aminobutyric acid type-A receptor agonist that temporarily inhibit the synaptic transmission. During reactivation, all groups equally expressed freezing. However, on Test A, MUS-treated group expressed significantly less freezing than VEH (VEH: 72 ± 3; MUS: 43 ± 7). After ten days, no spontaneous recovery was observed (VEH: 67 ± 5; MUS: 33 ± 5). One day after the spontaneous recovery test, the animals were submitted to an extinction session (VEH: 23 ± 2; MUS: 18 ± 5) and on the next day received a mild footshock in the context C. As a result, reinstatement could be observed in VEH-treated group, with increased fear response (VEH: 42 ± 5) while MUS-treated animals presented low levels of freezing (MUS: 23 ± 4), confirming that PL inactivation impaired the reconsolidation. When the reactivation was omitted and the PL was inactivated, no significant reduction of freezing was observed in the Test A. Further, to verify whether PL could be one of the neural substrates for the CBD effect, CBD (10 mg/kg) was systemically administered after the blockade of cannabinoid type-1 (CB1) receptors in PL with AM251 (50 pmol/side). Confirming a previous result, CBD blocked the reconsolidation step (VEH-VEH: 75 ± 9; VEH-CBD: 40 ± 9). This effect was abolished by the PL CB1 blockade (AM251-VEH: 71 ± 9; AM251-CBD: 70 ± 10). Altogether, the present work suggests that PL activity subserves fear memory reconsolidation and its CB1 receptors mediate the disruptive effects of CBD on this process.

Disclosures: C.J. Stern: None. **L. Gazarini:** None. **M.S. Hames:** None. **A.W. Zuardi:** None. **R.N. Takahashi:** None. **L.J. Bertoglio:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.02/KKK22

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

CAPES

FAPESC

Title: Comparing the role of α1- and β-adrenoceptors in consolidation and reconsolidation of adaptive and inappropriate contextual fear memories

Authors: *L. GAZARINI, C. A. J. STERN, A. P. CAROBREZ, L. J. BERTOGLIO; Farmacologia, Univ. Federal De Santa Catarina, Florianopolis, Brazil

Abstract: It has been shown that α1- and β-adrenoceptors have a distinct contribution during consolidation and reconsolidation of a weak and adaptive fear memory. For strong fear memories, the noradrenergic enhancement of consolidation or reconsolidation might underlie the formation of inappropriate memory traces, but it is unknown whether that outcome would recruit a different subset of adrenoceptors. Using the α2-adrenergic antagonist yohimbine to increase brain noradrenaline release, the present study sought to compare the relative contribution of α1- and β-adrenergic receptors to consolidate and reconsolidate contextual fear memories of different magnitude in rats. The consolidation protocol consisted of familiarization to the context A on day 1, a weak or strong training session (1 or 3 0.7 mA, 3 s footshocks) in the familiarized context on day 2, a re-exposure to that context (Test A) on day 3, and an exposure to a neutral context (Test B) on day 4. In the reconsolidation protocol, a 3-min session to retrieve/reactivate the memory trace was added 24 h before Test A. Freezing behavior was measured as an index of fear memory. The pretreatment with the α1-adrenergic antagonist prazosin (PRAZ; 0.5 mg/kg), the β-adrenergic antagonist propranolol (PROP; 10 mg/kg) or vehicle took place after the training (consolidation) or reactivation (reconsolidation) sessions. Yohimbine (YOH; 1 mg/kg) or vehicle (VEH) was given 10 min later. As observed during test A, the yohimbine-induced noradrenergic enhancement facilitates both the memory consolidation (VEH/VEH: 25 ± 3 %; VEH/YOH: 73 ± 7%) and reconsolidation (VEH/VEH: 24 ± 2 %; VEH/YOH: 55 ± 5 %) of a weak training. While propranolol prevented such effect in consolidation (PROP/YOH: 32 ± 2 %) and reconsolidation (PROP/YOH: 29 ± 3 %), prazosin was only able to prevent it during reconsolidation (PRAZ/YOH: 35 ± 2 %). Yohimbine caused fear generalization, as observed in

test B, when given during consolidation (VEH/VEH: 19 ± 3 %; VEH/YOH: 41 ± 6 %) or reconsolidation (VEH/VEH: 17 ± 2 %; VEH/YOH: 43 ± 4 %) of a strong training only. This effect was prevented by propranolol, but not prazosin, when given during consolidation (PROP/YOH: 20 ± 3 %) or reconsolidation (PROP/YOH: 13 ± 2 %). Altogether, present results highlight that while α1- and β-adrenergic receptors are differentially required during the consolidation and reconsolidation of an adaptive fear memory, there appears to be a preferential role of β-adrenergic receptors for the formation of inappropriate memories that subsides fear generalization.

Disclosures: L. Gazarini: None. **C.A.J. Stern:** None. **A.P. Carobrez:** None. **L.J. Bertoglio:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.03/KKK23

Topic: F.02. Animal Cognition and Behavior

Title: Fear extinction and reinstatement are dependent on distinct changes of cellular basis in medial prefrontal cortex

Authors: *N. IMAMURA, Y. MIURA, C. TESHIROGI, H. SHEN, N. MATSUKI, H. NOMURA;
Lab. of Chem. Pharmacol. Grad. Sch. of Pharmaceut. Sci., Tokyo Prefecture, Japan

Abstract: Anxiety disorder is a blanket term covering several different forms of a type of mental illness of abnormal and pathological fear and anxiety. In some cases, despite successful reduction of fear through exposure psychotherapy, inappropriate fear responses reappear with passage of time, presumably caused by re-exposure to the stimulus or stress. In contrast with extensive studies on the neuronal circuitry and neurochemical mechanisms leading to fear acquisition and extinction, few studies have focused on fear reinstatement. In this study, we investigated neuronal circuitry of reinstatement. Mice were conditioned with footshocks in a chamber, and 1 day later, they were re-exposed to the chamber without footshocks for 40 min. This long-term re-exposure, extinction training, extinguished conditioned fear responses. After that, they received a weak immediate shock in a different chamber, and then, fear responses were measured in the original conditioning chamber. As a result, the conditioned fear was reinstated. c-Fos immunostaining study revealed that an exposure to the conditioned chamber activated infralimbic (IL) region of medial prefrontal cortex (mPFC) after extinction training but not after

reinstatement. Microinjection of an NMDA receptor antagonist APV into mPFC before the weak immediate shock attenuated reinstatement. To investigate cellular basis of reinstatement, patch-clamp recordings were made from IL neurons. Extinction training elevated intrinsic excitability, whereas fear reinstatement was followed by reduction of mEPSC frequency. These results suggest that fear reinstatement as well as extinction is dependent on plasticity of IL neurons, but they are dependent on distinct changes of cellular basis in IL.

Disclosures: N. Imamura: None. **Y. Miura:** None. **C. Teshirogi:** None. **H. Shen:** None. **N. Matsuki:** None. **H. Nomura:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.04/KKK24

Topic: F.02. Animal Cognition and Behavior

Title: Peripheral stress impairs acquisition of fear memories via vagal afferents

Authors: *E. TOMIKAWA, H. NOMURA, N. MATSUKI;
pharmaceutical, The Univ. of Tokyo, Tokyo, Japan

Abstract: It is known that peripheral activities such as stress can affect learning and memory but exact mechanism is still unknown. In this study we used lipopolysaccharide (LPS) to induce abdominal discomforts in mice. LPS (250 ug/kg, i.p.) attenuated learning of inhibitory avoidance task tested the next day. Since LPS is known to stimulate the vagal afferents, the same task was tested in vagotomized mice. The LPS-induced suppression was completely abolished in vagotomized animals, indicating that peripheral information via vagal afferents is enough to affect hippocampus-dependent learning. To elucidate the mechanism in which vagal afferents impair memory formation, we analyzed c-Fos expression after LPS injection. As expected, LPS caused massive induction of c-Fos expression at the nucleus tractus of solitalius. Chronic electrical stimulation is clinically used to treat epilepsy and depression, and we have shown that electrical stimulation of vagal afferents augmented the perforant pass-CA3 neurotransmission via activation of the locus coeruleus (Shen et al. 2012). Therefore, LPS-induced activation of vagal afferents may affect hippocampal activities different from that of direct electrical stimulation.

Disclosures: E. Tomikawa: None. **H. Nomura:** None. **N. Matsuki:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.05/KKK25

Topic: F.02. Animal Cognition and Behavior

Support: Grant-in-Aid for Young Scientists (B)

Grant-in-Aid for Scientific Research on Innovative Areas, "Mesoscopic Neurocircuitry"

Mochida Memorial Foundation for Medical and Pharmaceutical Research

SENSHIN Medical Research Foundation

Astellas Foundation for Research on Metabolic Disorders

Title: The frontal association cortex is critical for formation but not retrieval of contextual fear memory

Authors: *H. NOMURA, D. NAKAYAMA, Z. BARAKI, A. NONAKA, N. MATSUKI;
Lab. Chem. Pharmacol, Grad Sch. Pharm, Univ. Tokyo, Tokyo, Japan

Abstract: The frontal association cortex (FrA) has reciprocal connections with the amygdala and is implicated in fear conditioning. The dendritic spines of FrA neurons are modified by fear learning and extinction. Inhibiting FrA activities after conditioning prevents the formation of the memory. However, the memory process in which the FrA is involved remains poorly understood. In this study, we examined the effect of inhibiting each memory process on contextual fear expression. Pre-conditioning infusion of APV, an NMDAR antagonist, or post-conditioning infusion of anisomycin, a protein synthesis inhibitor, into the mouse FrA impaired freezing behavior that was measured 1 day later. Pre-test infusion of muscimol, a GABAA agonist, into the FrA had no effect on the freezing behavior that was measured 1 day or 30 days after conditioning. Infusion of muscimol into the FrA had also no effect on the locomotor activity. These findings indicate that the FrA is involved in formation but not retrieval of contextual fear memory.

Disclosures: H. Nomura: None. **D. Nakayama:** None. **Z. Baraki:** None. **A. Nonaka:** None. **N. Matsuki:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.06/KKK26

Topic: F.02. Animal Cognition and Behavior

Title: Late Arc/Arg3.1 synthesis after retrieval is necessary for persistence of contextual fear memory

Authors: *D. NAKAYAMA, Y. YAMASAKI, N. MATSUKI, H. NOMURA;
7-3-1, Lab. of Chem. Pharmacol., Tokyo, Japan

Abstract: Memory retention requires long-term maintenance. Retrieved memory undergoes stabilization via de novo protein synthesis around the time of retrieval. However, the mechanisms underlying stabilization at
longer delayed time have not been explored. The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is an immediate early gene that has been widely believed to play a role in synaptic plasticity. Here, we show late Arc/Arg3.1 synthesis contributing to persistence of reactivated fear memory. Arc/Arg3.1 synthesis in the basolateral amygdala (BLA) was examined at several time points after retrieval of contextual fear memory. The western blotting showed biphasic increase in Arc/Arg3.1 synthesis. Arc/Arg3.1 synthesis increased at 2 and 12 h after retrieval. Late Arc/Arg3.1 synthesis was not increased when mice did not learn the association between context and footshock. To examine the role of late Arc/Arg3.1 synthesis in memory retention, *Arc/Arg3.1* antisense oligodeoxynucleotide, an Arc/Arg3.1 translation selective inhibitor, was infused into the basolateral amygdala. Inhibition of late Arc/Arg3.1 synthesis attenuated fear memory 7 days later, but not 2 days later, suggesting that late Arc/Arg3.1 synthesis is required for memory persistence. Mice showed intact fear memory when mice received *Arc/Arg3.1* antisense in the absence of the retrieval session. These data indicate that late Arc/Arg3.1 synthesis is necessary for persistence of reactivated fear memory.

Disclosures: D. Nakayama: None. **Y. Yamasaki:** None. **N. Matsuki:** None. **H. Nomura:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.07/KKK27

Topic: F.02. Animal Cognition and Behavior

Title: Measuring hippocampal gene expression in NFIL3 Knock-Out mice during consolidation and reconsolidation using CAGE sequencing

Authors: *L. R. VAN DER KALLEN[1], M. M. LENSELINK[1], I. M. C. BAKKER[2], L. M. PARDO CORTES[2], L. S. VIJFHUIZEN[3], A. M. J. M. VAN DEN MAAGDENBERG[3], S. SPIJKER[1], A. B. SMIT[1], R. E. VAN KESTEREN[1];
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Abstract: Long term changes in connectivity between neurons in the hippocampus are necessary for memory formation, and are regulated by transcription factors (TFs) such as CREB. NFIL3 is a TF related to CREB, and we previously showed that NFIL3 provides negative feedforward inhibition of CREB target gene expression in the context of regeneration after nerve injury. Moreover, NFIL3 is upregulated in hippocampal neurons by both CREB activation in vitro and fear conditioning in vivo. We hypothesize that NFIL3 and CREB co-operate to regulate gene expression in the hippocampus, which is necessary to make the long term changes in neuronal connectivity that are required for memory formation. We subjected wild-type (WT) or NFIL3-Knock-Out (KO) mice to fear conditioning, and measured context-dependent freezing responses 24 hours later. Freezing levels were subsequently also measured in a neutral context to measure generalized fear. Next we measured gene expression during fear conditioning induced consolidation and reconsolidation by Cap Analysis of Gene Expression (CAGE)-sequencing. Groups differed on 4 levels: 1) we compared WT and KO mice, 2) we measured during consolidation or reconsolidation, 3) we measured at 0, 0.5 and 6 hours after stimulation, and 4) we used mice that received the shock immediately upon entering the conditioning cage instead of after a 3 minute delay as a negative control group. In the immediate shock condition it has been shown that no memory is formed. We show that NFIL3 KO mice show comparable freezing levels when tested in the shock compartment, but increased freezing when subsequently tested in a neutral compartment, indicating increased generalized fear or decreased behavioral flexibility. CAGE sequencing data are currently being analyzed as to determine the molecular underpinnings of this behavioral deficit. Increased generalized fear or decreased behavioral flexibility in NFIL3 KO mice might be explained by the hypothesized role of NFIL3 as a negative regulator of plasticity gene expression. Lack of NFIL3 would lead to prolonged activation of plasticity genes, resulting in the formation of persistent or incorrect associations. The CAGE sequencing experiments will offer new insight into altered cascades of transcriptional activation in NFIL3 KO mice during memory processes with a resolution that is only recently available. Additionally we can tease apart gene activation that is specific to shock effects and memory processes, as well as consolidation or reconsolidation specific effects.

Disclosures: L.R. Van Der Kallen: None. **M.M. Lenselink:** None. **S. Spijker:** None. **R.E. van Kesteren:** None. **A.B. Smit:** None. **I.M.C. Bakker:** None. **L.M. Pardo Cortes:** None. **L.S. Vijfhuizen:** None. **A.M.J.M. van den Maagdenberg:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.08/KKK28

Topic: F.02. Animal Cognition and Behavior

Title: A role for amyloid-beta in memory stabilization

Authors: *P. S. FINNIE, J. PERDRIZET, K. NADER;
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Abstract: The accumulation of Amyloid beta (Abeta) in the brain has been strongly implicated in the pathogenesis of Alzheimer's Disease (AD). However, it has been proposed that this peptide might also serve important functions in normal brain physiology. Abeta levels have been observed to increase in response to neuronal stimulation and following acute or chronic stress, and may even facilitate memory consolidation. Furthermore, accumulating evidence indicates that elevated Abeta may influence the subunit composition of N-methyl-D-aspartate (NMDA) receptors, potentially lowering the NR2B:NR2A ratio. Given the well-documented function of reduced GluN2B:GluN2A ratio in closing developmental critical periods in sensory cortices, we theorized that endogenous Abeta could potentially contribute to metaplastic processes throughout the brain. Here we evaluated the role of Abeta in the stabilization of auditory fear memories. We observed that manipulating Abeta levels during fear conditioning can modulate the susceptibility of these memories to subsequently enter a labile state following their reactivation. These findings suggest that Abeta may contribute to the stabilization of consolidating memories against their future modification. An extension of this idea would predict that AD could be at least partially initiated by a change to how and when experience-dependent synaptic plasticity will occur. Our findings also highlight that unidimensional measures typically used to assess mnemonic ability in animal studies of Abeta and AD may obscure important aspects of this pathology.

Disclosures: P.S. Finnie: None. **J. Perdrizet:** None. **K. Nader:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.09/KKK29

Topic: F.02. Animal Cognition and Behavior

Title: Investigating the mechanism of memory reconsolidation in fear associated memories

Authors: S. BHATTACHARYA[1], W. KIMBLE[2], D. BHATTACHARYA[1], M. BUABEID[1], A. ALHOWAIL[1], M. DHANASEKARAN[1], M. ESCOBAR[2], *V. D. SUPPIRAMANIAM[1]; [1]Harrison Sc Pharm., Auburn Univ., AUBURN, AL; [2]Dept. of Psychology, Auburn Univ., Auburn, AL

Abstract: Retrieval of a previously-acquired memory renders it 'labile' until the memory is once again consolidated. This reconsolidation process has received much attention in recent years because labile memories may be prone to permanent alteration. Although much is known about the cellular processes that underlie reconsolidation, there are no available models of how synaptic plasticity changes through the reconsolidation period. Using rodents in conditioned fear preparations (retrieval, Rtv group), we investigated how synaptic plasticity change as a function of time through the reconsolidation period.We investigated changes in LTP, as well as the associated changes in glutamate receptor expression on a time-dependent manner through the reconsolidation period. Our data suggests that field Excitatory Post synaptic Potential (fEPSP) slopes are decreased in Schaffer Collateral pathway of the hippocampus in 1h post-Rtv animals (110.8+/-5%, p<.001, n=8) and improved in 4h post-retrieval animals (140.6+/-6%, p<.001, n=8) compared to controls. In 7h post-retrieval animals, the slopes corresponded to that of the control animals (180+/-10%, p<.001, n=8). A similar but more pronounced trend was seen in Dentate Gyrus recordings in the behaviorally intervened animals (n=8), which might be due to direct fear inputs from lateral amygdala to DG. Expression of N-Methyl-D-Aspartate (NMDA) and α-Amino-3-hydroxy-5-Methyl-4-isoxazole-Propionic Acid (AMPA) receptor subunits NR1, NR2A, NR2B, GluR1/2/3 were assessedat different time points/regions of the brain. GluR1 and NR1 subunits were downregulated in 1h animals, but returned to normal at 4h in the hippocampus and cerebellum. GluR2 subunits of AMPARs were downregulated in 1h animals in the hippocampus but not in cerebellum. Surprisingly, NR2B levels were above normal in 1h animals and decreased to control levels in 4h animals in all of the three brain regions. This effect might be due to synaptic NR2Bs role in memory destabilization. We further quantified synaptic receptor expression by using immuno-precipitated Post Synaptic Density 95 (PSD95) fraction. The trend was more distinct in PSD95 fractions of NR2B/GluR1in hippocampus. These results indicate a time dependent synaptic receptor expression pattern during fear input related memory reconsolidation. Currently, we are investigating the relationship between changes in synaptic plasticity and overt behavior.Successful accomplishment of the project will lead to betterment of

quality of life for Post Traumatic Stress Syndrome (PTSD) patients through selective erasure of fear-associated memories.

Disclosures: S. Bhattacharya: None. **W. Kimble:** None. **D. Bhattacharya:** None. **A. Alhowail:** None. **M. Escobar:** None. **V.D. Suppiramaniam:** None. **M. Buabeid:** None. **M. Dhanasekaran:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.10/KKK30

Topic: F.02. Animal Cognition and Behavior

Support: FONCYT PICT 2049, Argentina

FONCYT PICT 1482, Argentina

UBACYT X198, Universidad de Buenos Aires

PIP 5466, Consejo Nacional de Investigaciones Científicas y Te´cnicas, Argentina

Title: Nuclear Factor κB-dependent histone acetylation of Camk2d gene is specifically involved in memory persistence

Authors: N. FEDERMAN[1], V. DE LA FUENTE[2], G. ZALCMAN[2], *A. ROMANO[2]; [1]Univ. of Buenos Aires, Buenos Aires, Argentina; [2]Univ. of Buenos Aires, FCEN, FBMC, Buenos Aires, Argentina

Abstract: Previously, we have demonstrated that histone acetylation is a specific molecular signature of enduring memories consolidation. To gain insight into the specific gene expression effect of the induction of this epigenetic mechanism, we studied the participation of Nuclear factor κB (NF-κB) transcription factor in the process of acetylation during novel object recognition (NOR) memory persistence. To address this question, we used three different strength of training for NOR task in mice: one group of animals received a weak training protocol which did not induce long-term memory (LTM); animals which received a standard training which lead to 24hs LTM, and the last group of animals received a strong training which induced 7 weeks LTM. We found that only after strong training, NF-κB inhibition impaired memory persistence and, concomitantly, prevented the induction of general H3 acetylation. To determine the level of histone acetylation in specific genome locations, we studied promoter regions of particular genes that are associated with neural plasticity and memory. We studied two

genes that codify important proteins involved in memory formation: Zif268 and CaMKII. Accordingly, we found an important increase in H3 acetylation at a specific NF-κB-regulated promoter region of the Camk2d gene, which was reversed by NF-κB inhibition. This H3 acetylation increment leads to δCaMKII mRNA induction 6h after strong training, but not after weaker training protocols. This result shows that δCaMKII expression is only induced during consolidation of persistent forms of NOR memory. Our work presents a molecular link between transcription factor activation, epigenetic mechanism, and late gene expression in the regulation of memory persistence.

Key words: memory consolidation, memory persistence, histone acetylation, NF-κB, CamKIId.

Disclosures: N. Federman: None. **V. de la Fuente:** None. **G. Zalcman:** None. **A. Romano:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.11/KKK31

Topic: F.02. Animal Cognition and Behavior

Support: NRSA grant T32NS007413

NIH grant R01MH087463

Title: Transcriptome analysis reveals differences in processes that regulate gene expression during memory consolidation and retrieval

Authors: *L. PEIXOTO[1], M. WIMMER[1], S. POPLAWSKI[1], N. R. ZHANG[2], T. ABEL[1]; [1]Biol., [2]Wharton Sch., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Long term memory reflects the persistent changes in the brain that result from learning. Molecular defects in synaptic function are thought to be responsible for cognitive disorders, although those defects are yet to be fully understood. Several findings using learning paradigms in mice (such as contextual fear conditioning) have shown that long term memory formation requires transcription and protein synthesis. Previous research has shown that this requirement is limited to specific time periods sensitive to administration of anisomycin (a protein synthesis inhibitor) in the hippocampus (a brain region critical for memory storage). Transcriptional changes in several genes (fos, arc, zif268, dusp1, nr4a1) have been associated with memory formation, but outside a limited set of genes, little agreement exist between current genome-wide studies of memory consolidation and no study before has examined all defined

sensitive periods. In this study gene expression was examined using whole genome microarrays before training and during the established critical periods: 30 min., 4 hrs and 12 hrs and 24 hrs after contextual fear conditioning and after memory retrieval in C57BL/6J mice. A similar time-course was performed at the same time points without the learning experience to model the effect of circadian time in gene expression patterns in the mouse hippocampus (n=9 for all samples). Principal component (PC) analysis reveals that the strongest influences in gene expression in the hippocampus are pheromone response and time-of day and likely explains the lack of agreement and power of previous studies. After PC normalization, our study shows that the biggest changes in gene expression happen 30 min after learning and after retrieval of memory. Up-regulated genes after acquisition and retrieval overlap greatly and are involved in transcriptional control, including known transcription factors fos, zif268, junB and nr4a1. This was verified by q-PCR of known genes induced at 30 minutes. Several novel genes induced after contextual fear conditioning were identified and confirmed by qPCR in an independent cohort of animals. Interestingly, genes down regulated at the first sensitive period in consolidation and retrieval do not overlap in functions. Memory consolidation down regulates chromatin assembly while retrieval down-regulates RNA processing. Almost no-transcriptional changes can be detected 4 and 12 hours after learning. In addition, our study revealed a novel role for specific microRNAs in memory formation as well as differences in microRNA regulation between consolidation and retrieval.

Disclosures: L. Peixoto: None. **M. Wimmer:** None. **S. Poplawski:** None. **N.R. Zhang:** None. **T. Abel:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.12/KKK32

Topic: F.02. Animal Cognition and Behavior

Title: Histone acetylation enhances hippocampus- or perirhinal cortex-dependent non-affective memory consolidation and retrieval

Authors: *F. D. DUTRA, A. CRESTANI, F. BOOS, J. HAUBRICH, R. SIERRA, J. DURAN, Q. ZANONA, F. SANTANA, L. FLORES, J. QUILLFELDT, L. DE OLIVEIRA ALVARES; Dept. de Biofísica, Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil

Abstract: Neuroplastic mechanisms, including memory, are widely influenced by epigenetic marks (changes on genetic content not involved with nucleotides sequences alterations).

Currently, studies show that histone acetylation is one of epigenetic mechanisms that more influence memory consolidation. However, these studies use mainly behavioral paradigms with affective components (aversive or appetitive). Our present study verified histone acetylation epigenetic mechanism involvement over hippocampus- or perirhinal cortex-dependent non-affective memory consolidation and retrieval. To test this hypothesis, we injected Trichostatin A (TSA, HDAC inhibitor) or Garcinol (HAT inhibitor) directly over male adults Wistar rats' hippocampus or perirhinal cortex, immediately after training or before test of spatial object recognition (SOR - hippocampus-dependent) or object recognition (OR - perirhinal cortex-independent) tasks. We observed that TSA-injected animals showed both, SOR and OR, memories consolidation enhancement, when compared with respective controls. When animals were injected with Garcinol, however, these memories' consolidation was strongly impaired. In another experiment, animals injected with TSA showed SOR and OR memory retrieval enhancement, while animals treated with Garcinol showed information retrieval impairment. H3K9K14 levels biochemical analysis, by western blot, corroborates our behavioral findings. Taken together, these data show that histone acetylation actively participate of non-affective memory consolidation process. Other studies comparing different non-affective memory protocols are unknown. Moreover, this is the first time that HDAC's and HAT's actions over memory consolidation are showed together, in the same study. Furthermore, we showed that there is no difference in histone acetylation occurrence over hippocampus or other brain regions during memory consolidation and retrieval. Finally, we observed that, surprisingly, histone acetylation seems to act also over memory retrieval. Epigenetic action over memory retrieval is very interesting - and unexpected - and opens a wide range of investigation, where more studies are needed.

Disclosures: F.D. Dutra: None. **A. Crestani:** None. **F. Boos:** None. **J. Haubrich:** None. **R. Sierra:** None. **J. Duran:** None. **Q. Zanona:** None. **F. Santana:** None. **L. Flores:** None. **J. Quillfeldt:** None. **L. de Oliveira Alvares:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.13/KKK33

Topic: F.02. Animal Cognition and Behavior

Support: CONACYT GRANT 155242

DGAPA-UNAM GRANT IN209413 PAPIIT

Title: Arc expression in the insular cortex is involved in the updating after retrieval of an aversive memory trace

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Abstract: The formation and storage of a memory trace is a dynamic process that involves molecular changes and depending on the type of information the memory trace can be reinforced or updated upon reactivation. Memories of taste stimuli are remarkably plastic since the ability to learn if a certain food is safe to eat or became toxic is vital. We are interested in looking at the expression of Arc/Arg3.1, one of the immediate early genes that have been reported to be involved in learning induced long-term synaptic plasticity. Arc/Arg3.1 specifically accumulates in the dendrites that exhibit synaptic activity, thereby marking neurons that bear memories, however some evidence indicate that the hedonic value of the information is determinant to engage Arc expression within different brain structures. We hypothesized that the expression of Arc in the insular cortex would be related to the formation of an appetitive taste memory trace during the acquisition stage and during an updating process. In this study, we assessed the effect of the knock-down of Arc/Arg3.1 by the intracortical administration of an antisense oligodeoxynucleotide (ODN) on male Wistar rats before the acquisition phase of an aversively conditioned taste (saccharin followed by LiCl to induce gastric malaise), and before the acquisition of an appetitive "safe to consume" taste (saccharin followed by NaCl). The decrease of Arc/Arg3.1 expression affected only the formation of the appetitive saccharin and had no effect on the aversive trace formation. Additionally, we assessed the effect of the ODN administration on the hedonic value shifting (updating) of the saccharin memory trace from aversive to safe and vice versa. The results suggest that not only the initial formation of a "safe to consume" taste requires Arc/Arg3.1 expression, but the updating from aversive to safe also involves upregulation of Arc/Arg3.1. Interestingly, we were able to make this dissociation in a brain structure that is activated during the formation of both aversive and appetitive taste memory traces, indicating that the specificity of the information value incorporated to the trace might have a molecular signature involving Arc/Arg3.1.

Disclosures: K.R. Guzman-Ramos: None. **A. Venkataraman:** None. **P. Morin:** None. **F. Bermudez-Rattoni:** None.

Poster

577. Molecular Mechanisms of Memory Reconsolidation and Retrieval

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.14/KKK34

Topic: F.02. Animal Cognition and Behavior

Support: National Basic Research Program of China (no. 2009CB522004)

Natural Science Foundation of China (nos. 81201032, 31230033, and 31070958)

Title: Rac in the basolateral amygdala is crucial for the reconsolidation of auditory fear memory in rats

Authors: *Z. DING, P. WU, W. ZHU, H. SHEN, L. LU;
Natl. Inst. On Drug Dependence, Beijing, China

Abstract: Background: Acute and chronic changes in the stress response are major characteristics of post-traumatic stress disorder (PTSD), manifested as a conditioned fear memory. It has been reported that disruption of fear memory contributes to ameliorate PTSD. Consolidated fear memories that are susceptible to be disrupted immediately after retrieval undergo a reconsolidation process to become persistent, Rac (Ras-related C3 botulinum toxin substrate) plays an important role in the acquisition and extinction of fear memory. We hypothesized that Rac in the amygdala is crucial for the consolidation and reconsolidation of auditory fear memory.
Methods: The auditory fear conditioning procedure (1 s, 3 footshock, 0.75 mA) were used to explore the role of Rac in the amygdala in the consolidation and reconsolidation of auditory fear memory. Immediately after auditory fear conditioning or reexposure to a single tone previously paired with footshock in another context, we microinjected NSC23766 (the specific inhibitor of Rac1, 10 μg/μl or 27μg/μl, 0.5 μl per side) into the BLA or CeA. Then, the auditory fear memory performance was tested 24 hours after the microinjection.
Results: Microinjection of NSC23766 into the basolateral amygdala (BLA) but not central nucleus of the amygdala (CeA) immediately after memory reactivation disrupted the reconsolidation of auditory fear memory. Microinjection of NSC23766 into the BLA or CeA after auditory fear memory training had no effect on the consolidation of auditory fear memory.
Conclusions: Our results indicate that Rac in the BLA is crucial for the reconsolidation of auditory Pavlovian fear memory.

Disclosures: Z. Ding: None. **P. Wu:** None. **W. Zhu:** None. **H. Shen:** None. **L. Lu:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.15/KKK35

Topic: F.02. Animal Cognition and Behavior

Support: ONR N00014-13-1-0205

Title: Memory consolidation and reconsolidation in starlings: Exploring the interaction between interference and sleep

Authors: *T. P. BRAWN, H. C. NUSBAUM, D. MARGOLIASH;
Univ. of Chicago, Chicago, IL

Abstract: Memory consolidation strengthens newly encoded memory traces and stabilizes them against interference, a process widely believed to benefit from sleep. Behavioral evidence of sleep-dependent memory consolidation is well established in human memory tasks but is limited in adult animals. We previously demonstrated sleep-dependent memory benefits in European starlings that were trained on a Go/No-Go task to classify pairs of 5-second segments of novel starling songs. Classification accuracy improved after retention intervals that included sleep but not after waking retention (Brawn et al., Journal of Neuroscience, 2010). Furthermore, learning two similar classification tasks (tasks A and B) interfered with each other during the day, resulting in performance impairments for both tasks after waking retention. Yet, performance on both tasks improved after sleep (Brawn et al., Psychological Science, 2013). Here we explore if the consolidated memories are resistant to interference encountered after sleep, or if post-consolidation retrieval makes memory once again labile and therefore susceptible to interference. Auditory classification performance of starlings was examined across three days, with interference on the second day. Starlings in the Control (no interference) condition only learned task A, whereas starlings in the interference conditions also learned task B either before (Early-Interference) or after (Late-Interference) being retested on task A on day 2. Each condition, as expected from our prior work, resulted in a performance improvement after the first night of sleep. Control condition performance remained stable across the second day and second night of sleep. Performance in the Early-Interference condition also remained stable across the second day but additionally showed enhanced performance after the second night of sleep. In contrast, performance in the Late-Interference condition decreased across the second day but recovered after sleep. The pattern of results suggests that the retrieval of task A made the memory of task A susceptible to subsequent interference. Furthermore, the learning of task B instantiated a second night of sleep consolidation for task A. These results demonstrate a robust system for memory consolidation and reconsolidation in European starlings. We are now incorporating EEG recordings and auditory forebrain multisite recordings into the behavioral paradigm to determine the sleep states and sleep-dependent changes in neural activity associated with sleep consolidation.

Disclosures: T.P. Brawn: None. **H.C. Nusbaum:** None. **D. Margoliash:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.16/KKK36

Topic: F.02. Animal Cognition and Behavior

Support: CONACYT 60478, 155242

DGAPA-UNAM IN216709

Title: Retrieval and reconsolidation of object recognition memory are independent processes in the perirhinal cortex

Authors: *I. BALDERAS, M. SANTOYO-ZEDILLO, C. RODRIGUEZ-ORTIZ, F. BERMUDEZ-RATTONI;
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Abstract: Reconsolidation refers to the destabilization/re-stabilization process upon memory reactivation. However, the parameters needed to induce reconsolidation remain unclear. Memory retrieval is held as requisite to initiate reconsolidation. Here we evaluated the dissociation between memory reactivation and its behavioral expression by transient pharmacological disruption of memory retrieval of object recognition memory in rats. To assess whether retrieval is indispensable to trigger reconsolidation, we injected CNQX in the perirhinal cortex to block retrieval of object recognition, and APV to impede reconsolidation. We observed that APV impaired reconsolidation in the absence of retrieval. Therefore, stored memory underwent reconsolidation even though it was not recalled. These results indicate molecular dissociation between retrieval and reconsolidation and that retrieval is a dispensable condition to undergo reconsolidation in the declarative memory model of object recognition memory.

Disclosures: I. Balderas: None. **M. Santoyo-Zedillo:** None. **C. Rodriguez-Ortiz:** None. **F. Bermudez-Rattoni:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.17/KKK37

Topic: F.02. Animal Cognition and Behavior

Support: BBSRC UK

Title: Effects of dopamine D1 receptor antagonism on the reconsolidation of contextual fear memory

Authors: *C. STEVENSON[1], J. L. C. LEE[2], J. P. VOIGT[1], F. C. HEATH[1];
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Abstract: Once encoded, memories can be updated through reconsolidation to maintain their relevance. This process entails retrieval-induced destablization of the memory followed by its restabilization. Memory updating is thought to involve prediction error signalling which, in turn, is associated with dopamine cell activity. However, the role of dopamine transmission in memory reconsolidation remains unclear. Here we investigated the effects of the D1 receptor antagonist SCH 23390 on the reconsolidation of contextual fear memory in rats. In Experiment 1, animals were subjected to contextual fear conditioning (4 shocks). The next day SCH 23390 was given systemically 30 min before or immediately after brief (2 min) memory reactivation. Post-reactivation long-term memory was tested one day (PR-LTM1) and again one week (PR-LTM2) later. Freezing behaviour during memory testing served as a measure of contextual fear. We found no effects of SCH 23390 given before or after memory reactivation on freezing during reactivation, PR-LTM1 or PR-LTM2 testing. These results suggest that D1 receptor signalling is not involved in the retrieval or post-reactivation restabilization of contextual fear memory. It is also possible that the lack of effect of SCH 23990 was related to potential boundary conditions associated with our behavioural parameters; using weaker conditioning or longer reactivation may have allowed SCH 23390 to affect reconsolidation. Thus, in Experiment 2, we examined the effects of the NMDA receptor antagonist MK-801 on the retrieval and post-reactivation restabilization of contextual fear memory with the same behavioural parameters used in Experiment 1. Rats were conditioned and the next day MK-801 was given systemically 30 min before memory reactivation. We found that MK-801 decreased freezing during reactivation, PR-LTM1 and PR-LTM2 testing. These results confirm previous findings showing that NMDA receptor antagonism disrupts the post-reactivation restabilization of contextual fear memory. They also suggest that the lack of effect of SCH 23390 shown in Experiment 1 was unlikely due to any boundary conditions related to the behavioural parameters used. Ongoing experiments are examining if SCH 23390 given before reactivation prevents the disruptive effects of MK-801 on post-reactivation restabilization; this would suggest that D1 receptor signalling plays a role in memory destabilization, in keeping with emerging evidence linking this reconsolidation process with prediction error signalling, dopamine transmission and memory updating.

Disclosures: C. Stevenson: None. **J.L.C. Lee:** None. **J.P. Voigt:** None. **F.C. Heath:** None.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.18/KKK38

Topic: F.02. Animal Cognition and Behavior

Title: Transcriptional repression of the S1P Receptor Gpr12 regulates memory

Authors: *D. G. WHEELER, D. ELOW, R. JOHNSON, C. O'CARROLL, J. LAPIRA, W. JIANG, R. BARIDO, R. PETROSKI, E. MASSARI, R. SCOTT, T. TULLY, M. PETERS; Dart NeuroScience, San Diego, CA

Abstract: Activation of *de novo* transcription of plasticity genes by behavioral experience is required for long-term memory. However, it is unknown if the opposing process, activity-dependent transcriptional repression, plays a role in memory consolidation. Here, we show that mRNA expression of the g-protein coupled receptor Gpr12 is suppressed by synaptic activity in cultured hippocampal neurons. We also show enhanced long-term memory in heterozygous Gpr12 knockout mice, supporting a modulatory role for Gpr12 down-regulation *in vivo*. Consistent with these observations, RNA interference-mediated knockdown of Gpr12 in the hippocampus is sufficient to enhance memory. Our results link activity-dependent transcriptional repression of Gpr12 to memory formation. We suggest that targeting Gpr12 by small molecule antagonists may be a treatment for memory associated disorders in humans.

Disclosures: D.G. Wheeler: A. Employment/Salary (full or part-time):; Dart Neuroscience. **D. Elow:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **R. Johnson:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **C. O'Carroll:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **J. Lapira:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **W. Jiang:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **R. Barido:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **R. Petroski:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **E. Massari:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **R. Scott:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **T. Tully:** A. Employment/Salary (full or part-time):; Dart Neuroscience. **M. Peters:** A. Employment/Salary (full or part-time):; Dart Neuroscience.

Poster

577. **Molecular Mechanisms of Memory Reconsolidation and Retrieval**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 577.19/KKK39

Topic: F.02. Animal Cognition and Behavior

Support: CAPES

CNPq

FAPEMIG

Title: Social memory persistence in socially isolated adult mice is supported by hippocampal neurogenesis

Authors: *G. S. PEREIRA[1], B. M. M. MONTEIRO[1], F. A. MOREIRA[2], A. R. MASSENSINI[1], M. F. D. MORAES[1];
[1]Physiol. and Biophysics, [2]Pharmacol., UFMG, Belo Horizonte, Brazil

Abstract: Social memory comprehends the information necessary to identify and recognize co-specifics and it is essential for many forms of social interactions. The social memory persistence is strongly modulated by animal's experiences. We previously showed that social isolation (SI) during adulthood impairs the social memory persistence and the enriched environment (EE) prevents this deficit. However, the mechanisms involved in the effects of SI and EE on social memory persistence are still unknown. We hypothesized that the mechanism by which SI and EE are affecting social memory persistence is through modulation of neurogenesis. To address this issue, adult mice were submitted to seven days of one of the following conditions: group-housed in standard (GH) or enriched environment (GH+EE); social isolation in standard (SI) or enriched environment (SI+EE). We found an increase in the number of newborn neurons in the dentate gyrus of the hippocampus (DG) and glomerular layer of the olfactory bulb (OB), in both GH+EE and SI+EE mice. However, the increasing of newborn neurons in the granule cell layer of the OB was restricted to GH+EE group. Furthermore, both SI and SI+EE groups showed less neurogenesis in the mitral layer of the OB. Interestingly, SI mice had a worse performance in the buried food-finding task compared to GH mice, which was improved by EE. However, the latencies reached by both SI and SI+EE to find a hidden piece of chocolate were higher than the observed in the GH groups. To further analyze if increasing neurogenesis is the mechanism by which the EE is recovering the social memory persistence in SI mice, we administered the mitotic inhibitor AraC or saline, directly into the lateral ventricle of SI+EE mice. We found that AraC treatment decreased cell proliferation in both DG and OB and impaired social memory persistence in SI+EE mice, strongly suggesting that neurogenesis is supporting social memory persistence in socially isolated mice.

Disclosures: G.S. Pereira: None. **B.M.M. Monteiro:** None. **F.A. Moreira:** None. **A.R. Massensini:** None. **M.F.D. Moraes:** None.

578**Poster**

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.01/KKK40

Topic: F.02. Animal Cognition and Behavior

Support: AFOSR FA9550-12-1-0018

Title: Megamap: Continuous attractor model for place cells representing large environments

Authors: *K. R. HEDRICK, K. ZHANG;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Several continuous attractor models have been proposed for the CA3 subregion of the hippocampus due to the prevalence of recurrent collaterals among its place cells. In their standard form, the models assume that each place cell has a single place field in a given environment. However, recent experiments conducted in large environments indicate that multiple fields is a fundamental characteristic of place cells, where the number of fields increases with the size of the environment. This characteristic may be crucial, as models with one field per cell are limited by the size of the environment, and there is no natural way to extend the attractor when the rat moves beyond the artificial boundaries of the cognitive map. Furthermore, continuous attractor models appear to be inconsistent with the partial remapping observed among place cells.

We propose that place cells form a *megamap*, or a continuous attractor in which each cell has multiple, irregularly spaced place fields within a large environment. When the simulated rat is stationary at any location, relatively weak external input drives the system to an attractor state in which a localized activity bump is centered at the rat's location. As the rat moves, this weak input moves the bump along the rat's trajectory. Unlike previous models, the attractor has no artificial boundaries and can be extended naturally to include contiguous regions using a supervised learning rule. Consistent with standard attractor models, a single activity bump emerges given competing input patterns. However, as the strength of cross-excitation increases due to interactions among multiple place field pairs, the behavior shifts to a normalization mode in which two input patterns of similar strength give rise to two stable activity bumps. The former mode enables the network to perform pattern separation, while the latter mode gives the network the propensity for partial remapping. Developing a continuous attractor model for place cells that applies to large enclosures and is consistent with partial remapping is an important step forward as attractor networks create a stable spatial framework on which non-spatial information can be encoded.

Disclosures: K.R. Hedrick: None. **K. Zhang:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.02/KKK41

Topic: F.02. Animal Cognition and Behavior

Title: Differential effects of diazepam, zolpidem and THIP on non-REM sleep neurophysiology in the rat

Authors: *F. KERSANTÉ, M. W. JONES;
Univ. of Bristol, Bristol, United Kingdom

Abstract: Coordination of cortical slow-wave activity (SWA), thalamocortical spindles and hippocampal sharp wave-ripples (SWR) is believed to enable consolidation of memories across hippocampus and neocortex during mammalian non-REM sleep. These oscillations are driven by interactions between principal glutamatergic neurons and local GABAergic interneurons. Activation of $GABA_A$ receptors by allosteric modulators can induce sleep, but the differential actions of drugs with varied $GABA_A$ receptor pharmacologies on sleep neurophysiology and function remain relatively unexplored.
We compared the effects of diazepam (non-selective benzodiazepine), zolpidem (non-benzodiazepine enhancer, selective for α1 subunit containing $GABA_A$ receptors) and THIP (an agonist of extrasynaptic δ containing - $GABA_A$ receptors) on SWA, spindles, SWR and the temporal inter-relationships between them. Adult male Lister hooded rats were implanted with either two 32-channel silicon probes spanning medial prefrontal cortex (mPFC) and dorsal hippocampus or 16-tetrode drives targeting mPFC and dorsal CA1. LFP/multiple single units were recorded for 90 min following intra-peritoneal injection of either diazepam (4 mg/kg), zolpidem (3 mg/kg), THIP (4 mg/kg) or their vehicles.
In mPFC, slow wave density and amplitude were reduced by diazepam but increased by zolpidem. THIP decreased slow wave density but had no effect on amplitude. Diazepam induced a slight increase in the amplitude of spindles but neither THIP nor zolpidem changed spindle occurrence or amplitude. Whilst neither zolpidem nor THIP impacted the temporal relationship between spindles and delta oscillations, diazepam delayed the increase in spindle power subsequent to slow wave DOWN to UP-state transition, suggesting a change in synchronisation between spindles and SWA.
In CA1, diazepam decreased the density and amplitude (by 25%) of ripples whilst zolpidem increased ripple density and amplitude (by 30 %). THIP had no effects on ripples. The effects of

the drugs on ripple-spindle correlations and single unit activity in the PrL and CA1 are currently under investigation.
These data highlight differential roles of $GABA_A$ subunits in sleep neurophysiology and suggest differential consequences of hypnotics for sleep-dependent memory consolidation.

Disclosures: F. Kersanté: None. **M.W. Jones:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.03/KKK42

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS-1146316

NSF Grant BCS-0745573

NIH Grant R01MH082819

NCRR Grant P51RR000165

ORIP Grant ODP51OD011132

Title: Recognition errors suggest quick familiarity and slow recollection in rhesus monkeys

Authors: *B. M. BASILE, R. R. HAMPTON;
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Abstract: One influential model of recognition posits two underlying memory processes: recollection, which is detailed but relatively slow, and familiarity, which is quick but lacks detail. Whether the hippocampus is necessary for both recollection and familiarity, or only for recollection, has been a topic of debate in the past decade, and animal models are needed to provide a definitive answer. Most of the evidence supporting this dual-process model in nonhumans has come from analyses of receiver operating characteristic (ROC) curves in rats, but whether ROC analyses can demonstrate dual processes has been repeatedly challenged. Here, we present independent converging evidence for the dual-process model from analyses of recognition errors made by rhesus monkeys (Macaca mulatta). Recognition choices were made in three different ways depending on processing duration. Short-latency errors were disproportionately false alarms to familiar lures, suggesting control by familiarity. Medium-latency responses were less likely to be false alarms and were more accurate, suggesting onset of

a recollective process that could correctly reject familiar lures. Long-latency responses were guesses. A response deadline sped responding and selectively increased false alarms, suggesting that limiting processing time weakened the contribution of recollection and strengthened the contribution of familiarity. Together, these findings suggest fast familiarity and slow recollection in monkeys, that monkeys use a "recollect to reject" strategy to countermand false familiarity, and that primate recognition performance is well-characterized by a dual-process model consisting of recollection and familiarity.

Disclosures: B.M. Basile: None. **R.R. Hampton:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.04/KKK43

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS 1150292

Title: Temporary inactivations of the hippocampus and prefrontal cortex impair memory for sequences of events

Authors: *C. R. QUIRK[1,2], T. A. ALLEN[1,2], N. J. FORTIN[1,2];
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Abstract: Memory for sequences of events is critical to episodic memory. However, the neurobiology of this form of sequence memory is poorly understood. Previous research has shown that lesions to the hippocampus or prefrontal cortex impair sequence memory. Additionally, we have reported that neurons in the hippocampus and prefrontal cortex code for sequences of events, representations that may underlie sequence memory. However, it is difficult to directly compare these lesion and neurophysiology studies as they involved markedly different behavioral paradigms. To address this, we used localized inactivations to examine the contributions of the hippocampus and prefrontal cortex in the same sequence task used in our previous neurophysiological experiments. On each trial, rats were presented with a sequence of four odors through an odor port. On most trials, odors were presented in the correct sequence (A-B-C-D). On probe trials, one odor was presented in an incorrect sequence position (e.g., A-B-D-D). Rats were required to hold in the odor port for 1sec if an odor was presented "in sequence," or withdraw prior to 1sec if an odor was presented "out of sequence." A water reward was given for correct responses. Sequence memory was demonstrated when rats correctly discriminated

between “in sequence” and “out of sequence” odors. After reaching criterion, rats were implanted with six cannula targeted at the hippocampus and prefrontal cortex. Infusions of fluorophore-conjugated muscimol, a $GABA_A$-agonist, were used to temporarily inactivate target regions. Inactivations of each brain region examined the individual contributions of the hippocampus and prefrontal cortex to sequence memory, while disconnection inactivations (i.e., ipsilateral or contralateral hippocampus and prefrontal cortex) examined potential functional interactions between the regions. Saline infusions were used as within-subjects controls on alternate days. Results show that inactivations of the hippocampus alone or prefrontal cortex alone impaired performance, indicating that both structures are critical to sequence memory. Further, disconnection inactivations also impaired performance suggesting that the hippocampus and the prefrontal cortex interact to support sequence memory. These results are consistent with the neural data previously recorded in the same task, which suggested that the hippocampus and prefrontal cortex provide complementary representations to support performance. Overall, these experiments strongly support the hypothesis that the hippocampus and prefrontal cortex are part of a system from which the memory for sequences of events emerges.

Disclosures: C.R. Quirk: None. **T.A. Allen:** None. **N.J. Fortin:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.05/KKK44

Topic: F.02. Animal Cognition and Behavior

Support: Ray Thomas Edwards Foundation

Epilepsy Foundation

Hellman Foundation

NRSA 1F32MH096526-01A1

Title: Pathological high frequency oscillations in a chronic model of temporal lobe epilepsy occur during movement-related theta oscillations

Authors: *L. A. EWELL[1], K. B. FISCHER[1], L. LIANG[1], S. LEUTGEB[1,2], J. K. LEUTGEB[1]; [1]Ctr. for Neural Circuits and Behavior, Div. of Biol. Sci., UC-San Diego, La Jolla, CA; [2]Kavli Inst. for Brain and Mind, Univ. of California, San Diego, CA

Abstract: In temporal lobe epilepsy, the hippocampal network generates several pathological activity patterns including ictal spikes, interictal spikes, and pathological high frequency oscillations (pHFOs). Of these, pHFOs have gained recent interest because they serve as biomarkers for seizure focus, and thus research has focused on the relation between pHFOs and seizure. However, very little is known about how they might impact hippocampal function between seizures. Furthermore, it is unclear whether pHFOs are intensified versions of normal HFOs (ripples) or if they are mechanistically distinct. We were interested in whether pHFOs would occur during periods defined by movement-related theta, which would distinguish them from normal ripples. Furthermore, if pHFOs occurred during movement, it is likely that they would recruit spiking at inappropriate locations and negatively impact spatial coding. To study these questions, male Wistar rats with chronic epilepsy (those that experienced > 2 spontaneous seizures, low dose kainite model) were implanted with an electrode assembly that consisted of 14 independently movable tetrodes. Animals were trained to forage in an open field while hippocampal local field potentials and CA1 single units were recorded. Of the 5 epileptic rats, 4 exhibited pHFOs (> 200 Hz) in the CA1 region. Collectively, 13 ten-minute foraging sessions with ongoing pHFOs (n = 1117 pHFO events) were recorded. First we confirmed that animals were moving when pHFOs were detected. The median running speed at the onset of pHFOs was 4.1 cm/s, and pHFOs occurred over a wide range of running speeds including periods when animals were running quickly. Next we measured power in the theta band (6 -12Hz) around pHFOs during high running speeds (>4 cm/s), testing whether pHFOs were preceded by the cessation of local circuit dynamics that generate the theta rhythm. Average theta power 500 ms before the onset of pHFOs did not change from baseline segments (mean ± SEM =-37±71, n = 13 sessions, paired t-test, n.s.), suggesting that pHFO generation is not preceded by disruptions in theta. Finally, we asked whether CA1 pyramidal cells were recruited when pHFOs occur during foraging. Of the 65 pyramidal cells recorded in 3 rats during foraging, 47 were active during pHFO epochs. For these cells, the probability of firing at least one spike during a pHFO event was 6 ± 1 % higher than before the pHFO, and 8 ± 2 % higher than after the pHFO ($p < 0.01$, $p < 0.001$, one-way ANOVA). Such aberrant spiking would likely lead to degradation of place coding by those neurons, and may partially account for memory impairment associated with temporal lobe epilepsy.

Disclosures: L.A. Ewell: None. **K.B. Fischer:** None. **L. Liang:** None. **S. Leutgeb:** None. **J.K. Leutgeb:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.06/KKK45

Topic: F.02. Animal Cognition and Behavior

Support: NIH postdoctoral NRSA FMH096531A to M.P.B

Whitehall Foundation #2012-0685

Ellison Medical Foundation AG-NS-0724-10

NIMH 1 R21 MH100354-01

Title: Optogenetic control of the hippocampal theta rhythm

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Abstract: Physiological studies suggest that theta oscillations temporally coordinate cell assemblies by virtue of segregation into different theta phases and theta cycles. Computational models have described how these processes may support spatial and episodic memory function. More recently, debate has centered on whether theta oscillations play a supporting role in generating the spatial code in entorhinal grid cells and hippocampal place cells. Thus far, most experimental evidence driving this debate is correlational. Moreover, current manipulations of theta oscillations rely mostly on pharmacological innervations that have poor temporal resolution. Here, we use an optogenetic strategy to target and control the medial septal area, which is thought to be the neural circuitry that functions as the 'pacemaker' of the theta rhythm, to gain precise temporal control of theta oscillations during behavior.
We used a cre-dependent viral vector (AAV1.EF1a.DIO.hChR2(H134R)-eYFP.WPRE.hGH) in transgenic mice (129p2-Pvalbtm1(cre)arbr/j) to obtain expression of channelrhodopsin selectively in parvalbumin positive interneurons in the medial septum. A fiber optic was positioned above the medial septum and an 'optetrode' (fiber optic surrounded by four recording tetrodes) was implanted above the right hippocampus. Once animals recovered from surgery, tetrodes were lowered into the hippocampus, and blue light was delivered through the fiber optic into the medial septum. As animals ran on a linear track, we found that rhythmic square-wave stimulation with blue light (50% duty cycle) directly controlled the frequency of hippocampal theta oscillations up to and exceeding 40 Hz. The endogenous theta rhythm was no longer apparent, and there was a 1 to 1 relationship between the light pulse frequency and local field potential frequency. Upon cessation of light stimulus, theta frequency immediately returned to its baseline frequency (~8 Hz) suggesting that endogenous septohippocampal circuit dynamics recovered instantaneously. In addition, we found that single brief light pulses (10 ms) caused a reliable phase reset to the trough of the theta cycle. This optogenetic strategy in freely behaving mice can be used to elucidate the role of theta oscillations in spatial coding of grid cells and place cells, and to test the role of theta oscillations in episodic and spatial memory tasks with fine temporal precision.

Disclosures: M.P. Brandon: None. **M.L. Donegan:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.07/KKK46

Topic: F.02. Animal Cognition and Behavior

Title: Role of hipppocampal AMPA receptors in the retrieval of long-term spontaneous object recognition memory

Authors: *E. TAKANO, K. YAMADA, Y. ICHITANI;
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Abstract: Research on the role of hippocampus (HPC) in object recognition memory has produced conflicting results. Previous studies using hippocampal lesions suggest the possibility that HPC is important for object recognition memory when the delay between the sample and test phases is long enough. Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors are one of the main excitatory neurotransmitter receptors. In this study, we examined the role of hippocampal AMPA receptors in the retrieval of long-term (up to 3 wk) spontaneous object recognition memory by using 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX)-disodium, an AMPA receptor antagonist.
Male Wistar-Imamichi rats, 8-9 wk old at the beginning of the experiment were used. On the first day of the sample phase, the rat was allowed to explore a pair of identical objects (A) in an open-field arena (90×90×45 cm) for 5 min repeatedly for five times. On the next day, another pair of objects (B) was used for the sample phase. After the delay (1 or 3 wk), novel object preference was assessed by comparing the amount time spent exploring the familiar versus novel objects in the test phase. Exploration of an object was defined as directing the nose to the object at a distance of <2 cm and/or touching it with the nose. The time spent exploring the novel and familiar objects was recorded for 5 min, and the first 2 min was analyzed. We calculated the discrimination ratio (DR), the time spent exploring the novel object divided by the total time spent exploring the objects. Test phase was conducted for 2 days, one day for object A (A vs. C) and the other day for object B (B vs. D). Rats received bilateral hippocampal injection of either phosphate buffered solution (PB) or NBQX (2.5 mM, 1 μL) 15 min before the test phase . For the microinjection, rats were implanted with guide cannulae in the dorsal HPC 1 wk before the test phase (3-wk delay group) or 1 wk before the sample phase (1-wk delay group). After these long-term tests, the same rats were then given two 24-h delay tests using different object pairs.

One test was for NBQX treatment, and the other was for PB treatment.
Under PB treatment, rats showed significantly higher level of DRs compared with chance level (50%) in both 1-wk (71%) and 3-wk delay (68%) groups. However, under NBQX treatment, DRs in both groups (57%, 52%) were not significantly different from chance level. NBQX also decreased DRs in 24-h delay test (54%) compared with PB treatment (74%).Results suggest that AMPA receptors in the dorsal hippocampus are important for retrieval process of spontaneous object recognition memory regardless of the length of delay from 1 day to 3 weeks.

Disclosures: E. Takano: None. **K. Yamada:** None. **Y. Ichitani:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.08/KKK47

Topic: F.02. Animal Cognition and Behavior

Support: The PEW Charitable Trusts

Ray Thomas Edwards Foundation

Walter F. Heiligenberg Professorship

NRSA 1F32MH096526-01A1

Title: Non-spatial computations throughout the longitudinal dentate gyrus axis in support of working memory performance

Authors: *V. C. PIATTI[1], E. HWAUN[1], L. A. EWELL[1], M. JOSIC[1], S. AHMADI[1,2], S. LUM[1], R. BRAR[1], S. LEUTGEB[1,3], J. K. LEUTGEB[1];
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Abstract: Inputs from the dentate granule cells to downstream CA3 pyramidal neurons are known to be necessary for rats to perform spatial working memory (WM) tasks, which incorporate a high degree of complexity. We have recently determined that WM performance is not selectively supported by distinct regions of the dentate gyrus (DG) along the longitudinal hippocampal axis (Piatti et al., SFN 2012). In these studies granule cells were selectively lesioned with colchicine. Only lesions with a 60 - 80% volume reduction of the granule cell layer along the entire longitudinal axis resulted in impaired spatial WM on an eight-arm radial maze

task. However, lesions with corresponding % volume reduction specific to the rostrodorsal DG (rdDG) or the caudoventral DG (cvDG) did not impair WM, suggesting that network computations of remaining dentate neurons were sufficient for WM regardless of the anatomical location of the remaining circuits. We therefore sought to determine the mechanisms by which any DG region can support WM. We first asked whether lesions restricted to either rdDG or cvDG are compensated for by increasing the number of active neurons in the remaining network during WM. We used the immediate early gene *c-fos* to label active granule neurons in each group (control, rdDG lesions, cvDG lesions). In rats with rdDG or cvDG lesions, stereological methods showed that the fraction of *c-fos* labeled granule neurons in the remaining DG regions did not change during WM (n = 4 animals per group, n.s.). WM is thus supported in animals with rdDG or cvDG lesions without the compensatory activation of cells, but rather with a network in which sparsity is retained. Next, we assessed whether rdDG and cvDG neurons had shared firing patterns such that the same neuronal computations could be supported by any region. Rats were pretrained to perform the eight-arm spatial WM task and were implanted with electrodes. Local field potentials and DG single units (n = 50) were recorded along the longitudinal axis. As expected, a fraction of cells (~30 %) in rdDG had precise place fields on the maze while spatial selectivity in cvDG was only found in a small proportion of cells (~10 %; 0 of 19 cells for analysis restricted to the granule cell layer). However, most DG cells (87 % in rdDG, including those with fields, and 85 % in cvDG) showed spikes at the reward location, where distinct changes in the local field potential were observed (see Ahmadi et al., SFN 2013). Reward-related rather than spatial computations are thus found along the entire longitudinal DG axis. The primary function of DG is thus not limited to spatial pattern separation but to also update the network with information that is encoded at reward locations.

Disclosures: V.C. Piatti: None. **E. Hwaun:** None. **L.A. Ewell:** None. **M. Josic:** None. **S. Lum:** None. **R. Brar:** None. **S. Leutgeb:** None. **J.K. Leutgeb:** None. **S. Ahmadi:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.09/KKK48

Topic: F.02. Animal Cognition and Behavior

Support: Ray Thomas Edwards Foundation

The PEW Charitable Trusts

Walter F. Heiligenberg Professorship

NRSA 1F32MH096526-01A1

NSF/BMBF German-US Collaboration CRCNS-IIS-1010463

Title: 4-Hz and beta oscillations coordinate dentate network activity in a dentate-dependent working memory task

Authors: *S. AHMADI[1,3], V. C. PIATTI[1], L. A. EWELL[1], S. LEUTGEB[1,2], J. K. LEUTGEB[1]; [1]Neurobiol. Section and Ctr. for Neural Circuits and Behavior, Div. of Biol. Sci., [2]Kavli Inst. for Brain and Mind, UCSD, La Jolla, CA; [3]Cognitive Sci. Ctr. Amsterdam, Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The hippocampus is critical for memory formation, including spatial working memory. Dentate circuits along the entire dorsoventral axis were found to be sufficient for supporting spatial working memory (Piatti et al., SFN 2013). The dentate gyrus is also known to be particularly important for memory encoding. We therefore asked how local field potentials and neuronal firing patterns change at reward sites, where information is updated during working memory. We used an 8-arm radial maze task to record local field potentials (LFPs) throughout the hippocampus and single units in the dentate gyrus (n = 6 rats). The first four arms were presented in random order by the experimenter, after which the animal was presented with all eight arms and could optimally solve the task by visiting the remaining four arms. We analyzed LFPs in three different bands, 4-Hz (2-5 Hz), theta (6-12 Hz) and beta (18-35 Hz). As expected, LFP power in the theta band decreased at the transition from running to stopping. Most dentate granule cells were strongly phase-locked to LFP theta oscillations. While theta decreased when rats stopped at reward sites, we detected an increase in power in the 4-Hz band (61.1% in dorsal DG, 20.7% in intermediate DG) and in the beta band (28.3% and 46.2%, respectively) at the same transition. The average increase did not differ between trials with and without errors. The peak increase in the beta band occurred earlier than the peak increase in the 4-Hz band. The increase in beta power was limited to recording sites in the dentate gyrus, while the increase in the 4-Hz band was found for all hippocampal recording sites and could thus coordinate firing patterns between subregions. We then asked whether the higher LFP power was indicative of increased coordination of neuronal firing patterns at reward sites. Most putative dentate granule cells showed significant phase coupling to 4-Hz and to beta, and the coupling was much higher at reward locations compared to elsewhere on the maze. Furthermore, phase locking was stronger in intermediate/ventral compared to dorsal DG. In addition to analyzing LFP power in working memory trials, we performed the same analyses on foraging trials with no memory demand. Without memory demand, fluctuations of LFP power were at chance levels (n.s.; shuffle test). The increases in LFP power in the 4-Hz and beta band and the phase locking of DG cells to these oscillations suggest that dentate circuits contribute to memory encoding at reward sites. The effects were observed throughout the dorsoventral axis and could thus reflect neural network dynamics for processing mnemonic rather than spatial information during working memory performance.

Disclosures: S. Ahmadi: None. **V.C. Piatti:** None. **L.A. Ewell:** None. **S. Leutgeb:** None. **J.K. Leutgeb:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.10/KKK49

Topic: F.02. Animal Cognition and Behavior

Support: ANR

Title: Role of astrocytes connexin in the regulation of sleep oscillatory pattern

Authors: *M. M. LACROIX[1], L. ROUX[2], C. GIAUME[3], K. BENCHENANE[1];
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Abstract: Coordination across brain structures is thought to be crucial for the appropriate consolidation of memory trace. Cortical slow oscillations modulate the timing of hippocampal sharp wave-ripples (SPW-Rs) occurrence which support neuronal reactivations. Hippocampal SPWR-s are then followed sequentially by cortical reactivation, cortical delta waves and finally cortical spindles.
Our project aims at challenging the classic view of the "neurocentric" concept in brain rhythms regulation during sleep. Indeed, recent evidences showed that astrocytes can regulate cortical slow oscillations during sleep and are involved in brain processes related to memory deficits induced by sleep deprivation, both mechanisms being mediated by the activation of A1 adenosine receptors.
One of the key feature of astrocytes is their expression of connexins that form either hemichannels (leading to the release of neuroactive molecules) or gap junction (making astrocytes organized into networks of communicating cells).
We therefore investigated the role of connexins in the fine regulation and coordination between hippocampal, cortical and thalamic oscillations during natural sleep, by multi-site recordings in wild-type mice or transgenic mice double knock-out for astrocytic connexins Cx43 and Cx30 (dKO).
Our results show that there is a massive decrease of slow oscillations during sleep in dKO mice in the olfactory bulb, confirming the results obtained in vitro by Lisa Roux, but also a change in the coordination between other brain structures oscillations.

Our results support the involvment of astrocytes in regulation of neuronal network functioning and brain oscillatory activity.

Disclosures: M.M. Lacroix: None. **K. Benchenane:** None. **L. Roux:** None. **C. Giaume:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.11/KKK50

Topic: F.02. Animal Cognition and Behavior

Support: BBSRC Grant BB/H002383/1

Alzheimer's Research UK Pilot Grant

Wellcome Trust OXION Studentship

Title: Hemispheric asymmetry of hippocampal memory processes in mice

Authors: *O. SHIPTON[1,3], J. APERGIS-SCHOUTE[3], D. BANNERMAN[2], K. DEISSEROTH[4], O. PAULSEN[3], M. KOHL[3];
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Abstract: To investigate the behavioural consequences of the recently-uncovered hemispheric asymmetry in long-term potentiation (Kohl et al. Nat. Neurosci. 2011), we used acute optogenetic silencing of CA3 pyramidal cells in the left or right mouse hippocampus during two hippocampus-dependent memory tasks. Mice received injections of a viral construct encoding halorhodopsin (eNpHR3.0: a light-sensitive chloride pump) under the control of the CaMKIIα promoter into the left or right CA3; this gave expression of eNpHR3.0 in excitatory CA3 neurons of one hemisphere and their projections to CA1 neurons both ipsilaterally and contralaterally. Control mice received injections of a viral construct that did not encode NpHR3.0. All mice had an optical fibre implanted on the injected hemisphere to illuminate the dorsal CA3 during behavioural testing. We found that unilateral silencing of only the left CA3 impairs the acquisition of a hippocampus-dependent spatial long-term memory task that requires multiple trials to learn: main effect of transgene $F(1,76) = 6.01$; $p = 0.017$; significant transgene by side interaction $F(1,76) = 11.46$; $p = 0.001$ due to significant effect of transgene on left side $F(1,76) = 16.62$; $p < 0.001$). As controls we used a hippocampus-dependent short-term memory task and

a hippocampus-independent visual discrimination task. Overall, our results showed a within-subject dissociation of hippocampal memory processes between hemispheres, with the left CA3 being required for a spatial long-term memory task. This suggests that mice may have differences between left and right CA3 in the information received, how it is processed and stored, or a combination of both.

Disclosures: O. Shipton: None. **J. Apergis-Schoute:** None. **D. Bannerman:** None. **K. Deisseroth:** None. **O. Paulsen:** None. **M. Kohl:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.12/KKK51

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS 1150292

NIA R01 AG034613

Title: A cross-species approach to investigating memory for sequences of events

Authors: *A. M. MORRIS[1,2], T. A. ALLEN[1,2], A. T. MATTFELD[3], C. E. L. STARK[1,2], N. J. FORTIN[1,2];
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Abstract: The ability to learn and remember sequences of events is a key feature of episodic memory shared by a variety of species including humans, non-human primates, and rodents. However, the neurobiological substrates of this form of sequence memory are not well understood. While rodent studies indicate the hippocampus and the prefrontal cortex are critical for this capacity, it has proven difficult to translate findings from animals to humans due to large differences in task demands across studies. Although progress has been made using complementary approaches, there is currently no single task that allows for direct comparisons across species. To address this important issue, we used an integrated cross-species approach to develop a sequence task designed to test the ability of humans and rats to learn and remember the order of serially experienced items. The task involved the presentation of sequences of items (fractal images for humans, pure chemical odorants for rodents) and required subjects to make a judgment as to whether items were presented “in sequence” or “out of sequence”. Probe trials were used to assess the cognitive strategies used by humans and rats supporting successful

sequence memory. Results show that rats and humans (1) were able to learn and remember arbitrary sequences at similar levels of accuracy, (2) displayed similar response times when making “in sequence” or “out of sequence” decisions, and (3) used similar cognitive strategies supporting sequence memory judgments. These findings strongly validate the human and rat sequence tasks as homologous, i.e. involving the same cognitive processes. This novel cross-species sequence memory task should prove useful in future investigations examining the neurobiology of the memory for sequences of events. In addition, preliminary data shows that the sequence task is sensitive to aging, and may therefore serve as a potential diagnostic tool for detecting cognitive dysfunction in older adults with age-related neurodegenerative diseases. This approach will also allow the use of rodent models of age-related neurodegenerative diseases to explore the cellular basis of the deficits seen in human patient populations.

Disclosures: A.M. Morris: None. **T.A. Allen:** None. **A.T. Mattfeld:** None. **C.E.L. Stark:** None. **N.J. Fortin:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.13/KKK52

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH082893

Title: Maternal exercise during pregnancy improves object recognition memory in adult male offspring

Authors: *A. M. ROBINSON, D. J. BUCCI;
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Abstract: Maternal exercise during pregnancy has been shown to be beneficial for the offspring. However, most studies have looked at the effect of maternal exercise on anxiety and spatial cognition in juvenile or adolescent pups. The present study was designed to test the effect of maternal exercise during pregnancy on recognition memory that relies on the perirhinal cortex (PER) in adult offspring. Male and female Long Evans rats were allowed to mate during a 72-hour period. Pregnant female rats were then assigned to exercise or non-exercise conditions. Rats in the exercise condition had 24 hr access to a running wheel inside their homecages during gestation and rats in the non-exercise condition were housed in cages without wheels. Once pups were born, the wheels were removed and pups stayed with their mothers until being weaned on PND 21. When they were 60 days old, object recognition memory was assessed in 10 male

offspring from exercising mothers and 11 male offspring from non-exercising mothers. After being habituated to the testing environment, rats were placed in a plastic tub for 5 min and allowed to explore two identical sample objects. Twenty-four hours later (test session), rats were returned to the tub, which contained one of the sample objects as well as a new, unfamiliar object and given 2 min to investigate the items. A discrimination ratio served as the dependent variable of interest and was calculated as the time spent exploring the novel object divided by total time spent exploring both objects. There were no group differences in time spent exploring the objects during the sample session ($p>0.15$). During the test session, however, the offspring of exercising mothers were able to successfully discriminate between novel and familiar objects ($p<0.03$) in that they spent more time exploring the novel object than the familiar object. The offspring of non-exercising mothers were not able to successfully discriminate between objects ($p>0.6$) and spent an equal amount of time with both objects. This result was obtained again when rats were tested two weeks later with a different set of objects. A subset of rats underwent a third object recognition test and were euthanized 1 hr later to assess c-FOS expression in the PER. There was a significant group difference in that the offspring of exercising mothers had more c-FOS expression in the PER than the offspring of non-exercising mothers ($p<0.006$). These results indicate that maternal exercise during pregnancy can improve object recognition memory that relies on the PER in male offspring and these effects last into adulthood.

Disclosures: A.M. Robinson: None. **D.J. Bucci:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.14/KKK53

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant R01-MH078821

NIH grant P50-MH58880

RIKEN Brain Science Institute

Title: Inception of a false memory in the hippocampus

Authors: *S. RAMIREZ[1], X. LIU[2], P.-A. LIN[2], J. SUH[2], M. PIGNATELLI[2], R. L. REDONDO[2], T. J. RYAN[2], S. TONEGAWA[2];
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Abstract: Memories are usually excellent guides for cognition and behavior. However, they can also be unreliable, and false memories in particular have had severe legal consequences. Despite these social ramifications, the lack of relevant animal models has largely hindered our understanding of the neural basis of false memories. Here, we describe the inception of a false memory in mice by optogenetically manipulating memory engram-bearing cells in the hippocampus. Mice were allowed to explore a particular environment and dentate gyrus (DG) or CA1 neurons activated by the exposure to this context were labeled with channelrhodopsin-2 (ChR2). These neurons were later optically reactivated during fear conditioning in a different context. The DG experimental group showed increased freezing in the original context in which a foot shock was never delivered compared to: the control groups not expressing ChR2, a group in which light was omitted, and a group that underwent a similar manipulation in CA1. The recall of this false memory was context-specific, activated similar downstream regions engaged during natural fear memory recall, and was also capable of driving an active fear response in a conditioned place avoidance paradigm. The formation of a false memory interfered with the concurrent formation of a natural memory and could have either an additive or competitive effect during the recall of the natural fear memory. Together, our data demonstrate that it is possible to substitute a natural conditioned stimulus (CS) with artificially reactivated DG cells that bear contextual memory engrams to incept an internally represented false fear memory.

Disclosures: S. Ramirez: None. **X. Liu:** None. **P. Lin:** None. **J. Suh:** None. **M. Pignatelli:** None. **R.L. Redondo:** None. **T.J. Ryan:** None. **S. Tonegawa:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.15/KKK54

Topic: F.02. Animal Cognition and Behavior

Support: National Institutes of Health Grant MH079511

Title: Burst phase offsets among hippocampal theta cells do not vary with position or movement trajectory: Implications for spatial coding by oscillatory interference

Authors: *R. M. DE GUZMAN, L. R. HALLADAY, H. T. BLAIR;
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Abstract: Oscillatory interference models hypothesize that spatially tuned neurons derive their positional firing properties by detecting location-specific synchrony among inputs from velocity-controlled theta oscillators (VCTOs).

Here, we recorded pairs of hippocampal theta cells to investigate whether they behaved like VCTOs. Theta cells were recorded from CA1, CA3, and dentate gyrus of the dorsal hippocampus as rats (n=4) foraged freely for food pellets in a cylindrical chamber measuring 80 cm in diameter. Each recording session was 120-180 min long. Theta cells were identified on the basis of high tonic firing rates (>10 Hz), low spatial information (<0.1 bits/spike), and a significant peak in the 6-9 Hz band of the spike train autocorrelogram's power spectrum. A total of 42 well-isolated theta cells were recorded simultaneously with at least one other theta cell, and 58 unique pairs of theta cells were stably held for >2.0 h. Directional burst frequency tuning curves (DBFTs) were plotted to estimate the vector along which each theta cell's burst frequency was modulated by movement direction. Most pairs of simultaneously recorded cells had similar DBFTs, and showed a similar correlation between burst frequency and running speed, implying that their preferred movement vectors were the same. Burst phase offsets between cell pairs were estimated from crosscorrelograms between their spike trains, by measuring deviation of the center peak from zero phase lag. Multiple crosscorrelograms were generated for each cell pair from spikes generated at different locations and movement trajectories. It was found that all 58 pairs of theta cells exhibited a rigidly stable burst phase offset from one another (which differed for each pair of cells), which did not change with the rat's position or movement trajectory. These findings suggest that if hippocampal theta cells do function as VCTOs, then they are a homogeneous population sharing the same preferred movement vector (thus encoding different phases of the same VCTO signal). Such theta cells could generate a globally synchronized inhibitory 'reference oscillation' in the hippocampus; individual place cells may be driven to fire when they receive excitatory inputs from VCTOs that are synchronized in antiphase with the global inhibitory reference.

Disclosures: R.M. De Guzman: None. **L.R. Halladay:** None. **H.T. Blair:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.16/KKK55

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant MH079971

WCU Program R31-10089

Title: Hippocampal subregional firing correlates for parametric alterations in distal cue-configuration in a goal-directed task

Authors: *I. LEE, PhD, S.-B. PARK;
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Abstract: Several studies have reported that, depending on the amount of changes induced to the distal cues in the environment, a place cell changes firing patterns in various ways (e.g., spatial remapping, rate modulation, etc.) presumably to reflect the environmental changes. It is still unknown, however, whether such physiological changes in the hippocampus directly relates to the animal's behavior when the rat is required to use those cues to make spatial choices in a goal-directed task. In order to test whether there are functional relationships between the amount of change in distal cues and the firing patterns of place cells related to spatial choice behavior, we recorded single units simultaneously from the hippocampus while rats (n=5) ran along a linear track at the end of which a choice should be made between two food wells. A correct choice-response was contingent upon the angular distance between two sets of visual cues with each set attached to a curtain (cue-curtain) that was movable along a circular curtain track in the ceiling. Three distinct visual cues were attached to each cue-curtain and the medial edges of the curtains were separated by one of the following angular distances: 0º, 14º, 66º, and 80º. Rats were initially trained in a standard task in which "standard cue-configurations" (i.e., 0º and 80º) were associated with the left and right food wells. Our previous study has demonstrated that the dorsal hippocampus is indispensable to normal performance in this task. Once trained to criterion, a hyperdrive was implanted and tetrodes were lowered to target regions while the rat was still being trained. Once tetrodes were all in the target regions, recording commenced in the standard task for several days. Afterwards, an ambiguous task began in which two "ambiguous cue-configurations" (i.e., 14º and 66º) were introduced in a session (randomly mixed with the standard cue-configurations and sharing reward contingencies of closer standard cue-configurations), and the single-unit recordings were made. Among well-isolated putative principal cells (n=1636), ~20% of cells exhibited place-specific firing patterns during outbound journeys in the behavioral sessions. Preliminary findings suggest that the centroid of a place field shifted backwards as the cue-curtains were separated from each other between trials. It appears that the significant separations of the field centroid associated with the 0º-configuration from the centroids associated with the other configurations (14º, 66º, and 80º) could explain performance differences between 0º cue-condition (~96%) and the others (79%, 87%, and 89%).

Disclosures: I. Lee: None. **S. Park:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.17/KKK56

Topic: F.02. Animal Cognition and Behavior

Support: 3DNeuroN project in the European Union's Seventh Framework Programme, Future and Emerging Technologies, grant agreement 296590

Title: Towards a fully flexible system for studying hybrid networks with controlled topology

Authors: *L. DEMKO, V. HOOP, A. POPERT, H. DERMUTZ, J. VOROS;
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Abstract: The use of cultured neuronal networks as a model for their in vivo counterparts allows researchers to study the central nervous system, especially the brain, in a controlled environment. Specific neuronal connectivity patterns in the brain are implicated to play a role in the perception, processing and storage of information, so the building of small neuronal networks with controlled topology is a promising approach to investigate the basic circuits of the nervous system. Realizing such bottom-up systems on multi-electrode arrays (MEA) makes a way to study the dynamics of functional neural networks systematically as a function of the network structure. Here, MEA chips provide an in-situ, bidirectional, non-invasive communication between the individual cells and the microelectrode array, and such localized neurons cultured on the electrodes can act as bio-friendly interfaces for collateral sprouts from an in vivo system. Several surface patterning techniques have been developed in order to control the neuronal connectivity, mostly based on surface chemistry in combination with micro- and nanostructuring. However, most of them have common issues with long-term compliance and clustering, and with these methods the modification of the desired topology involves complex preparatory processes. Our first goal was to develop an inexpensive, flexible, and easy to use setup which is compatible with all the previous patterning solutions, but having extra features such as co-culturing and the ability to probe the system by localized mechanical or chemical stimulus. The main part of the setup is a special hollow cantilever for the FluidFM system. This cantilever enables patterning of selective coatings on cell culture substrates with promoters or inhibitors of cell adhesion and growth, seeding of the cells directly onto the electrodes, and local stimulation - all in a fully controlled, but completely flexible manner. The control over the number and type(s) of seeded cells, local concentrations of specific biochemicals combined with mechanical and electrical stimulation, and the recording of neural activity in networks with predefined topology give platform for the analytical testing of pharmaceuticals and the development of biosensors.
The present work focuses on building of hybrid networks, in which functional neurons interact with artificial model ones, in order to prove, evaluate, and fine tune the models we have for memory and learning. With the results, we aim to provide insights into the role of neural and synaptic properties such as synapse development, plasticity and connectivity, and extract the algorithms that support neurocomputation in brain tissue.

Disclosures: L. Demko: None. **V. Hoop:** None. **A. Popert:** None. **H. Dermutz:** None. **J. Voros:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.18/KKK57

Topic: F.02. Animal Cognition and Behavior

Support: Geman Research Foundation (DFG, SFB 874/B3).

Title: Spatial olfactory learning modifies place field formation in the hippocampus

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Abstract: Spatial encoding in the hippocampus is multifactorial, and it is well established that metric information about space is conferred by place cells that fire when an animal finds itself in a specific environmental location. Visuospatial contexts comprise a key element in the formation of place fields. Nevertheless, hippocampus does not only use visual cues to generate spatial representations. In the absence of visual input, both humans and all other vertebrate species studied in this context, to date, are capable of generating very effective spatial representations. However little is known about the relationship between non-visual sensory modalities and the establishment of place fields. Substantial evidence exists that olfactory information can be used to learn spatial contexts. Here, we recorded hippocampal pyramidal neurons in the CA1 region by means of single unit recordings while rats were navigating in a circular arena in darkness where distinct odors were hidden and diffused from multiple locations from below the floor. We observed that learning about a distinct odor constellation in an environment where visual and auditory cues are suppressed, results in global remapping in about one-third of cells by shifting their place fields completely and in about one-third of cells by switching place fields on or off. It also results in more confined and stable place fields. These place fields rotate when the odor constellations are rotated and remap when the odor constellations are shuffled. These data support that the hippocampus can use non-visuospatial resources, and specifically spatial olfactory information, to modify spatial representations.

Disclosures: S. Zhang: None. **D. Manahan-Vaughan:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.19/KKK58

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS-1146316

NSF Grant BCS-0745573

NIH Grant RO1MH082819

NCRR Grant P51RR000165

ORIP Grant P51OD011132

Title: Mechanisms for transitive inference in monkeys (Macaca mulatta)

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Abstract: Rhesus monkeys live in social groups in which dominance ranks are transitive. If Monkey A is dominant to Monkey B, and Monkey B is dominant to Monkey C, then the fact that Monkey A is dominant to Monkey C can be correctly inferred via transitive inference. The importance of social dominance in these animals may make them especially good models for studying the cognitive processes involved in transitive inference. Monkeys were trained with 6 pairs of overlapping image discriminations (A+B-, B+C-, C+D-, D+E-,E+F-, F+G-.) and correctly selected the higher ranked item in probe tests with non-adjacent novel pairings (e.g. BD, CF). We performed tests to determine the extent to which these choices were controlled by associative values and inferred relations. We measured the associative values of the 7 TI images using concurrent RI-RI schedules and found that these values did not correlate with choice in transitive inference tests. Monkeys linked two previously learned 7-item lists into one 14-item list after training with a single linking pair. Together these findings indicate that implied order explains most transitive inference choices in monkeys. Such relational processing is a more viable mechanism for transitive inference based on observational learning of social dominance relationships, where explicit reinforcement is not present.

Disclosures: R.R. Hampton: None. **R.P. Gazes:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.20/KKK59

Topic: F.02. Animal Cognition and Behavior

Support: Ray Thomas Edwards Foundation

Epilepsy Foundation

Hellman Foundation

NIH NS35915

Title: Interneurons robustly and consistently increase their firing rates during the minutes preceding behavioral seizures in a chronic model of temporal lobe epilepsy

Authors: *L. LIANG[1], L. A. EWELL[1], C. ARMSTRONG[3], I. SOLTESZ[3], S. LEUTGEB[1,2], J. K. LEUTGEB[1];
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Abstract: Seizures reflect abnormal synchronized activity of a neuronal network, however, the activity dynamics preceding seizure onset are still poorly understood and algorithms for seizure prediction typically rely on local field potential recordings. Recent research has asked whether single-unit recordings might improve the predictability of seizures. Neuronal activity was found to change inconsistently before behavioral seizure onset such that an increase in variability was observed in the minutes before the onset. GABAergic interneurons integrate excitatory inputs from local and afferent networks. Such convergence of input may allow interneurons to be more sensitive to widespread neural synchrony that leads to seizures compared to principal cells. We asked whether interneurons might be more reliable predictors of seizures than principal cells. To record activity patterns of interneurons and principal cells before behavioral seizures, we used the repeated low-dose kainate model of chronic temporal lobe epilepsy in male Wistar rats. We implanted animals that developed epilepsy ($\geq$ 2 spontaneous seizures) with tetrode arrays and video monitored the rats (n=3) while local field potentials and single unit activity were recorded from CA1 and CA3. We identified a total of 20 behavioral seizures (rat 1: 4 seizures across 4 days, rat 2: 5 seizures across 3 days, rat 3: 11 seizures across 5 days). We assessed the activity patterns of hippocampal pyramidal cells (CA1, n=79 cells; CA3, n=34 cells) and interneurons (CA1, n=13 cells; CA3/DG, n=18 cells) during the 5 minutes preceding the behavioral seizure onset. First, we characterized baseline firing rates during 100 second epochs. We found that only ~10.8% of principal cells exhibited firing rates that deviated by more than 3 standard deviations from the baseline in the minute before the seizure onset, whereas ~50% of interneurons deviated from baseline up to 2 minutes before seizure onset. The average increase in firing rate of all interneurons was +60% during the 2 minutes before the seizure onset compared to baseline. Interneurons are thus a much better predictor of seizures during the minutes before the seizure

onset. Because of the large effect size, even small numbers of recorded interneurons can reliably predict a seizure. Of the cells that were tracked across ≥ 2 seizures (n=14 principal cells and 8 interneurons in two rats), 31 of 32 showed a consistent change in firing rate preceding seizure onset. Together, these data suggest that knowing the change in firing from a previous seizure improves prediction, but that the gains from using interneurons for detection algorithms would be much more robust.

Disclosures: L. Liang: None. **L.A. Ewell:** None. **C. Armstrong:** None. **I. Soltesz:** None. **S. Leutgeb:** None. **J.K. Leutgeb:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.21/KKK60

Topic: F.02. Animal Cognition and Behavior

Support: Ellison Medical Foundation (AG-NS-0724-10)

National Institute of Aging P50 AG005131

Neuroplasticity of Aging Training Grant 2 T32 AG00216-21

Title: Pre-plaque amyloid pathology causes robust changes in hippocampal function, which does not further deteriorate upon plaque formation

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Abstract: The robust accumulation of amyloid-beta (Aβ) plaque pathology that occurs late in Alzheimer's disease (AD) progression has long been viewed as the cause of memory loss in the disease. However, recent research has indicated that synaptic toxicity in the AD brain is caused by soluble forms of Aβ and begins well before insoluble Aβ plaques accumulate. Many current Alzheimer's transgenic models accumulate plaque pathology at such an accelerated rate that it is difficult to study physiological changes across the transition from the pre-plaque soluble Aβ stage to the plaque Aβ stage. Indeed, many current models accumulate Aβ so robustly that the pre-plaque stage can only be investigated in juvenile or young adult animals. Here, we used a rat model of Alzheimer's disease (McGill-Thy1-APP) which shows a 15 month long pre-plaque

stage, in order to compare hippocampal firing patterns before and after plaque formation. Our previous research has shown that changes in place field properties preceded plaque formation in this model. Place fields became large, diffuse, and unstable over testing at 6 and 12 months of age. Changes in place fields occurred before measurable changes in mnemonic behavior, as assessed by Morris Water Maze testing at 6 and 12 months of age. Because we have confirmed that plaques did not accumulate for 15 months in these animals, these changes in behavior and place cell function are caused by soluble Aβ species rather than by plaques. When the onset of plaque pathology occurs (at approximately 15-18 months of age) we observed that deposits accumulated throughout the hippocampus, septum, and entorhinal cortex, as well as other cortical areas. We now ask whether the substantial deterioration of hippocampal function during the pre-plaque stage will further progress after Aβ plaques appear. In this set of experiments, chronic recordings of CA1 pyramidal cells and of hippocampal local field potentials were performed in freely behaving 20-24 month old homozygous rats and littermate controls. In these animals, the deterioration in spatial firing was not any more pronounced than in 12 month old animals, despite the advanced progression of Aβ plaque pathology at the later time point. We are currently testing whether local field potentials also show pathological changes during the pre-plaque stage without a further decline at later stages. The pre-plaque dysfunction in spatial firing indicates a major deterioration of hippocampal system function through the effects of soluble Aβ assemblies rather than of insoluble Aβ plaques. The most substantial changes in hippocampal function may therefore occur early in the progression of Aβ pathology and of Alzheimer's disease.

Disclosures: A.L. Blackshear: None. **J. Davis:** None. **M.B. Martinez:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.22/KKK61

Topic: F.02. Animal Cognition and Behavior

Support: Ray Thomas Edwards Foundation

Walter F. Heiligenberg Professorship

NSF/NIH/Bundesministerium für Bildung and Forschung grant 101046

Alberta Innovates – Health Solutions

Title: Hippocampal subregions CA2 and CA3 relay complementary information about temporal and spatial context to CA1

Authors: *E. A. MANKIN[1,2], F. T. SPARKS[4], G. W. DIEHL[1], S. LEUTGEB[2,3], J. K. LEUTGEB[2];
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Abstract: In many situations the ability to estimate the recency of a remembered event is critically important for guiding behavior, yet the sensory content of the experience alone cannot always provide information about when it occurred. How temporal information might be incorporated into memory has long been a topic of interest in psychology, and one class of computational models has suggested that the comparison or combination of two signals--one stable and one time-varying--may allow the recency of an event to be computed. To address the question of how recency may be represented in episodic memories, we recorded the activity of neurons in hippocampal subregions CA1, CA2, and CA3 while rats randomly foraged in highly familiar square and circular enclosures in the morning and, after an interval of 6 hours, in the afternoon for multiple days. This behavioral paradigm allowed us to compare the neuronal activity patterns for repetitions of the same environment to determine whether they changed over time, as well as to determine to what extent similar contexts were discriminated. We have shown that the representation of identical events remains highly stable in CA3 for at least 30 hours, while representations in CA1 change gradually with time [Mankin et al., PNAS, 2012]. This suggests the possibility that CA1 is playing the hypothesized role of computing elapsed time by comparing a stable memory signal from CA3 with a time-varying signal from another source. We asked whether CA2 might be a source of time-varying information to CA1. We found that the population representation of identical events in CA2 changed markedly with time, with the mean population vector correlation decreasing from 0.59 for sessions less than 1 hour apart to 0.11 for sessions recorded 30 hours apart. The change in activity patterns as a function of time was more pronounced in the CA2 cell population than in either CA1 or CA3 (CA2 vs CA1: $t(14) = 5.7$, $P<0.001$, CA2 vs CA3: $t(14) = 8.4$, $P<0.001$). While representations in CA2 changed rapidly with passing time, we did not observe a difference between distinct shapes any greater than the difference between identical events at any time interval. Our data therefore show that CA2 is the only hippocampal subregion that does not discriminate between similar spatial contexts in the same location but shows only a time-varying spatial firing pattern. Thus it appears that CA2 codes strongly for elapsed time and minimally for spatial context, while CA3 codes strongly for spatial context and minimally for elapsed time. Because CA2 and CA3 inputs converge in CA1, this identifies CA1 as a brain region that can compute elapsed time and integrate the when, what, and where aspects of episodic memory.

Disclosures: E.A. Mankin: None. **G.W. Diehl:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None. **F.T. Sparks:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.23/KKK62

Topic: F.02. Animal Cognition and Behavior

Support: HHMI

NARSAD

Title: A cortico-hippocampal learning rule enhances information flow through the hippocampal circuit by shaping local inhibitory microcircuit activity

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Abstract: How does coordinated activity between distinct brain regions implement a set of learning rules to sculpt information processing in a given neural circuit? Using interneuron cell-type specific optical activation and pharmacogenetic silencing in vitro, we show that temporally precise pairing of direct entorhinal perforant path (PP) and trisynaptic Schaffer collateral (SC) inputs to CA1 pyramidal cells selectively suppresses SC-associated perisomatic inhibition from cholecystokinin (CCK) expressing interneurons. The CCK interneurons provide a surprisingly strong feedforward inhibitory drive to effectively control the coincident excitation of CA1 pyramidal neurons by convergent inputs. Thus, in-phase cortico-hippocampal activity provides a powerful heterosynaptic learning rule for long-term gating of information flow through the hippocampal excitatory macrocircuit by silencing the CCK inhibitory microcircuit. Studies in progress complement these findings, by exploring how inhibition can shape the integration of timed synaptic inputs to implement temporally precise mnemonic codes.

Disclosures: J. Basu: None. **S.A. Siegelbaum:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.24/KKK63

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant P50MH094263

Title: Long-range projections of medial prefrontal cortex and hippocampus revealed through whole tissue imaging

Authors: *A. J. DIMAURO[1], W. A. LIBERTI, III[2], R. J. ROBINSON, II[1], D. J. SHEEHAN[1], J. M. GAUTHIER[3], H. EICHENBAUM[1];
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Abstract: Connections between the rodent medial prefrontal cortex (mPFC) and hippocampus are critical for several learning and memory functions including associative learning tasks (Lee & Solivan, 2008), strategy switching (Rich and Shapiro 2007) and working memory (Churchwell and Kesner 2011), but detailed anatomical information remains elusive, due to the difficulty of using contemporary methods. Previous studies have shown that mPFC receives unidirectional projections from area CA1 of the ventral hippocampus (Hoover & Vertes, 2007) but mPFC projects only indirectly back to the hippocampus. This connection is largely through the nucleus reuniens of the thalamus, an area that also receives input from the hippocampus (Vertes et al 2007). To enhance our understanding of the functional relevancy of mPFC and hippocampal interactions, the precise geometry of these neural projections must be resolved. We show that tissue clearing allows for whole and partial brain imaging at high spatial resolution, while preserving fine cellular structure, even at the spinal level.

Disclosures: A.J. DiMauro: None. **W.A. Liberti:** None. **R.J. Robinson:** None. **D.J. Sheehan:** None. **J.M. Gauthier:** None. **H. Eichenbaum:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.25/KKK64

Topic: F.02. Animal Cognition and Behavior

Support: STW AET7613 ‘MICROMAX'

EU FP7 grant 270108 “Goal Leaders”

PROJECT ENLIGHTENMENT 284801

Title: Single-trial analysis of place field properties in control and CA1 NMDAR1-KO mice

Authors: *H. O. CABRAL[1,2,3], C. FOUQUET[4], M. VINCK[2,3], L. RONDI-REIG[4], C. M. A. PENNARTZ[2,3], F. P. BATTAGLIA[5,6,2,3,7];
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Abstract: Hippocampal place cells, neurons that selectively respond to animal position, show impaired function upon a range of manipulations affecting CA1, e.g. disrupting inputs{Brun2008}, abolition of self-motion information {Terrazas2005} and NMDA receptor deletion {McHugh1996, Korotkova2010}. These studies, however, report impaired place field (PF) properties averaged over an entire recording session, but how the instantaneous spatially-related activity is affected is still unknown.
We recorded CA1 place cell activity in control(CTR) and CA1 NMDAR1(NR1)-KO mice shuttling on a circular track and analyzed place field properties on a trial-by-trial basis. Similar to previous studies {McHugh1996}, session-averaged PFs of NR1-KO mice were larger, however when the PF was calculated on a trial-by-trial basis, no difference was observed. Furthermore, we show that NR1-KO PCs ('Place Cells') present a markedly increased inter-trial variability of its firing maps, which explains the larger session-averaged PF size and suggests that the larger PF size is a consequence of a deficit of a PC to consistently spike at the same location across passages. By analyzing the directionality and symmetry, two measures of how much PC firing is driven by distal and local cues/path integration, respectively, we report that NR1-KO are impaired in both, hinting at impairments in hippocampal spatial processing.
Spatial representation in CA1 NR1-KO mice improved, however, with experience on the track, such that it was at the CTR level towards the end of the recordings, possibly due to synaptic plasticity in structures afferent to CA1, (which are unaffected in these mice) and their influence on CA1 activity.
Finally, we show that beta(17-23), low(23-40) and high(55-95) gamma frequency oscillations decrease as mice proceed from the first to the last track traversal, an effect only reminiscently present in NR1-KOs. This effect was present throughout recordings and may parallel a decreased alertness as a session progresses.
These results detail single-trial PC patterns of activity in mice and clarify the causes underlying spatial representation deficits caused by the absence of NMDARs in CA1. We show that, rather than simply having larger PFs, NR1-KO PCs fail to properly integrate different inputs, reflecting allocentric and path integrating processes, which lead to increased jitter across trials.

Disclosures: H.O. Cabral: None. **C. Fouquet:** None. **L. Rondi-Reig:** None. **M. Vinck:** None. **C.M.A. Pennartz:** None. **F.P. Battaglia:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.26/KKK65

Topic: F.02. Animal Cognition and Behavior

Title: Parallels between individual variability in hippocampal NR2a expression and Morris Water Maze learning

Authors: J. CHO[1], L. SMITH[3], J. FLINN[2], D. BURDETT[2], *R. F. ACKERMANN[4], S. E. BACHUS[2];
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Abstract: We have previously found correlations between hippocampal NR2a receptor expression and Morris Water Maze (MWM) learning indices in rat pups (Smith et al., SfN Abstr., 2004), and between nucleus accumbens (NA) D3 receptor expression and MWM learning in their dams (Burdett et al., SfN Abstr., 2010). Here we have explored hippocampal NR2a mRNA levels in the same dams (n=16), and investigated correlations between the pups and their dams.
Spatial reference learning and memory testing was conducted utilizing the submersible Atlantis-style platform. The platform remained stationary 1cm below the surface of the water in the North-West quadrant throughout the testing period, except during probe trials. Testing consisted of three 60 second trials per day over eight days, with 45 seconds between trials. Spatial reference memory was assessed by performance on probe trials conducted every sixth trial. During probe trials, the platform was lowered to the bottom of the pool so that it was undetectable by the rats. Time spent in the target quadrant and number of platform crossings were measured. In situ hybridization histochemistry was used to measure NR2a mRNA levels in dentate gyrus, CA3, CA2, and CA1 in sections from postmortem brains.
NA D3 mRNA was highly positively correlated with hippocampal NR2a mRNA across the dams ($p<.02$). Significant negative correlations between CA2 NR2a and time spent in the correct quadrant on the third trial of days 2-7, for both probe and non-probe trials, were replicated in the dams ($p<.05$). Platform crossings during the final probe trial were also highly negatively correlated with NR2a mRNA throughout the hippocampus ($p<.03$). However, hippocampal NR2a was not significantly correlated between dams and their pups. These data add to evidence implicating NMDA receptor composition in individual variability in learning ability, but argue against heritability of this factor.

Disclosures: J. Cho: None. **L. Smith:** None. **J. Flinn:** None. **D. Burdett:** None. **R.F. Ackermann:** None. **S.E. Bachus:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.27/KKK66

Topic: F.02. Animal Cognition and Behavior

Support: EU GOAL LEADERS 270108

Title: Within and between area replay in Hippocampus, Barrel Cortex and Perirhinal cortex

Authors: *J. J. BOS, M. VINCK, A. B. VAN MOURIK-DONGA, C. M. A. PENNARTZ; Swammerdam Inst. for Life Sciences, Cogn NeuroSci, Cognition Systems Neurosci., Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The brain has a strong hierarchical architecture where information from primary sensory areas is integrated into complex and multimodal sensory representations in the medial temporal lobe and hippocampal formation. We are interested in how sequences of spiking activity in these different brain structures are temporally organized and pre- or replayed during rest/sleep activity. To address this question, we performed simultaneous tetrode recordings from four different brain areas in the awake behaving rat. These four areas comprised the primary visual, barrel, perirhinal cortex, and the dorsal CA1 field of the hippocampus. From each of these four areas, spikes and Local Field Potentials were recorded with eight tetrodes per area. Across these four areas, we recorded >1500 well isolated single units from four rats performing a visual discrimination task. A target (CS+) and distractor stimulus were presented simultaneously on two screens, flanking the two arms of a figure-8 maze. When the rat selected the arm where the target stimulus was presented, a pellet reward was obtained at the end of the arm. Each arm contained additional tactile cues, coupled to reward amount. Four implanted rats performed ~60 trials with on average ~75% correct performance per session. The task period was flanked by pre- and post sleep/rest sessions. We examined reactivation of task related activity in the pre and post sleep phases during periods of slow wave sleep, ripples and spindles. We first analyzed reactivation using explained variance measures to assess similarities in pairwise correlation patterns. Reactivation occurred both within and between different brain areas. In particular, strong reactivation was observed between pairs of cell in hippocampus, perirhinal, and barrel cortex. This occurred both within/around ripple periods and outside ripple periods. In conclusion,

medial temporal lobe structures display a clear offline information trafficking with sensory neocortex that recapitulates previous behavioral experiences.

Disclosures: J.J. Bos: None. **M. Vinck:** None. **A.B. van Mourik-Donga:** None. **C.M.A. Pennartz:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.28/KKK67

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS 1150292

NIMH P50MH071702

NIMH MH068982

NIMH MH60013

NIDA DA16454

The Feinberg Foundation

Title: Neural representations of sequences of events in the hippocampus parallel behavioral performance

Authors: *T. A. ALLEN[1,2], D. M. SALZ[3], S. A. MCKENZIE[3], M. E. HASSELMO[3], H. B. EICHENBAUM[3], N. J. FORTIN[1,2];
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Abstract: Memory for sequences of events is a key feature of episodic memory. Prior studies have shown that lesions to the hippocampus impair this form of sequence memory, but the underlying neural mechanisms that contribute to this capacity remain unclear. In a previous report, we showed that CA1 neurons coded for many aspects of an odor sequence task, including neurons that differentiated between odors presented “in sequence” and “out of sequence” (sequence cells). However, the link between these neural representations and performance on the task remains unclear. Here we directly compared hippocampal representations of the sequences of odors between sessions in which animals showed poor (i.e., first session on a specific

sequence) or strong sequence memory (i.e., when they performed at asymptotic levels). In the task, rats were trained to remember a sequence of five odors presented in a single port at the end of a straight alley maze. Odors were either presented in the correct ordinal position ("in sequence;" e.g., A-B-C-D-E), or one odor was presented out of position ("out of sequence;" e.g., A-B-D-D-E). Rats were required to hold in the odor port for 1sec if an odor was presented "in sequence," or withdraw prior to 1sec if an odor was presented "out of sequence." Sequence memory was demonstrated when rats correctly responded to both "in sequence" and "out of sequence" odors. We recorded 235 CA1 neurons in sessions that showed strong sequence memory ("in sequence" accuracy: 88%; "out of sequence" accuracy: 70%). We report that 42% of neurons showed odor selectivity, 46% showed ordinal selectivity, and 33% were sequence cells. Importantly, many of these neurons were responsive to specific combinations of odor and position, a type of conjunctive representation not previously reported. We also recorded 191 CA1 neurons from the same rats during sessions in which they showed poor sequence memory (i.e., the first session on a specific sequence; "in sequence" accuracy: 88%; "out of sequence" accuracy: 23%). We report that the proportion of responsive neurons significantly dropped in all categories: 25% showed odor selectivity, 26% ordinal selectivity, and 11% were sequence cells. These proportions are lower than in strong sequence memory sessions, but higher than expected by chance, presumably because the rats are in the process of learning the sequences. Notably, the proportion of sequence cells recorded in individual rats was positively correlated with sequence memory performance across the sessions ($r = 0.7$, $p < .05$). These results suggest that sequence coding in the hippocampus is directly related to memory for sequences of events.

Disclosures: T.A. Allen: None. **S.A. McKenzie:** None. **M.E. Hasselmo:** None. **H.B. Eichenbaum:** None. **N.J. Fortin:** None. **D.M. Salz:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.29/KKK68

Topic: F.02. Animal Cognition and Behavior

Support: NSF/BMBF German-US Collaboration CRCNS-IIS-1010463

Boehringer Ingelheim Fonds PhD Fellowship to M. I. S.

Ellison Medical Foundation AG-NS-0724-10

NIMH 1 R21 MH100354-01

Title: The medial entorhinal cortex is required for hippocampal phase precession

Authors: *M. I. SCHLESIGER[1,4], C. C. CANNOVA[1], E. A. MANKIN[1], B. B. BOUBLIL[1], J. B. HALES[2], J. K. LEUTGEB[1], C. LEIBOLD[5], S. LEUTGEB[1,3];
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Abstract: The average theta oscillation cycle of individual hippocampal cells is shorter than the theta cycle of the local field potential, which gives rise to phase precession and may enable spike-timing dependent plasticity. Theta phase-precession is observed in all hippocampal subareas and in the medial entorhinal cortex (MEC). Computational models suggest that phase precession either originates in one brain region and propagates through the circuit or that it can be generated locally in multiple subregions within the circuit. It has previously been shown that entorhinal phase precession does not require the hippocampus. To determine whether phase precession can independently arise in the hippocampus and in MEC or whether hippocampal phase precession requires MEC inputs, we recorded hippocampal neuronal activity in rats with bilateral excitotoxic MEC lesions. Place fields were larger and less frequent after the lesion, but the average and peak firing rates of the remaining place fields were comparable to those of control rats. Furthermore, single CA1 cells remained modulated by the theta rhythm although with reduced modulation depth (control mean ± SEM: 25.5 ± 1.58, MEC lesion: 13.8 ± 0.98). Because we found that spatial firing and theta modulation, which are prerequisites for hippocampal phase precession, were preserved, we examined whether this would imply that the spike timing within theta cycles is also preserved after MEC lesions. In control animals, robust phase precession was found with circular-linear regression analysis when pooling repeated passes through each place field during running in a box for 10 minutes (significant precession was detected in 46 % of the fields). The average slope was less than zero in controls ($p < 0.001$, $t = -3.69$), but not in MEC lesioned rats (n.s., $t = 0.026$). The loss of phase precession for pooled data in the MEC lesion group could arise while phase coordination between cells is intact, but when the starting phase with respect to LFP oscillations varies from pass to pass. To examine this possibility, we analyzed phase precession during single passes. For single passes in controls, we found robust phase precession ($p < 0.001$, $t = -23.89$). In the lesion group, the average slope for single passes was not different from zero (n.s., $t = 1.62$), indicating a loss of phase precession. This pattern of results was also observed on the linear track. We thus find that MEC lesions abolish hippocampal theta-phase precession in 1D and 2D environments, independent of whether single-pass data or pooled data were analyzed. MEC is thus not only a source of spatial information to hippocampus but also provides temporal coordination for hippocampal firing patterns.

Disclosures: M.I. Schlesiger: None. **C.C. Cannova:** None. **E.A. Mankin:** None. **B.B. Boublil:** None. **J.B. Hales:** None. **J.K. Leutgeb:** None. **C. Leibold:** None. **S. Leutgeb:** None.

Poster

578. **Animal Learning and Memory: Cortical and Hippocampal Circuits III**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 578.30/KKK69

Topic: F.02. Animal Cognition and Behavior

Title: The role of the hippocampus and the avian "prefrontal cortex" for extinction learning and renewal of appetitive conditioning

Authors: *D. LENGERSDORF, M. STÜTTGEN, O. GÜNTÜRKÜN;
Dept. of Biopsychology, Ruhr-Universität Bochum, Bochum, Germany

Abstract: Research on the extinction of conditioned fear in rodents has implicated prefrontal cortex and hippocampus in context-specific extinction learning. However, to elucidate fundamental mechanisms of learning and refine extinction-based behavior therapy e.g. for drug addiction and addictive gambling, learning under appetitive reinforcement conditions needs to be investigated.
Therefore, we devised an appetitive conditioning within-subject paradigm to investigate context-specific extinction learning. Pigeons acquired responses to a rewarded conditioned stimulus (CS) in context A. Once a defined performance criterion was reached, responding to the CS was extinguished in a different context B. Subsequently responding to the CS was tested in both the context of acquisition (ABA renewal) and in the context of extinction (ABB). Importantly, responding to another CS was acquired in context B, extinguished in context A, and also tested for renewal in both contexts. This within-subject version of ABA renewal thus allows testing each subject in an ABA as well as in an ABB design for direct comparison. In order to characterize neural substrates for extinction learning, we inactivated hippocampus via local administration of tetrodotoxin (TTX) into both hemispheres. The drug was administered via intracerebral cannulas immediately before extinction training. Hippocampal inactivation did not significantly affect responding during extinction or on the test for renewal but led to increased spontaneous recovery. Further experiments employing NMDA receptor blockade and stimulation in the nidopallium caudolaterale, the presumed functional analogue of mammalian prefrontal cortex, are currently underway.

Disclosures: D. Lengersdorf: None. **M. Stüttgen:** None. **O. Güntürkün:** None.

579**Poster**

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.01/KKK70

Topic: F.02. Animal Cognition and Behavior

Support: GlaxoSmithKline funded

Title: Resveratrol and forced exercise prove beneficial against both neurological and metabolic deficits associated with ageing

Authors: *R. C. HUSSEY, S. M. O'MARA;
Trinity Col. Inst. of Neurosci., Dublin, Ireland

Abstract: The leading risk factor for neurodegeneration is ageing. There are many theories as to precisely what aspects of ageing are causal of this negative effect on cognition and brain function. To date, this answer remains unclear. With whole-body metabolism also naturally decreasing as we age, it seems probable that these parallel deficits may not be entirely dissociated from one another. A wealth of research shows that increasing metabolism has beneficial effects on neurodegeneration, both in prevention and rehabilitation; most likely through increased cAMP levels affecting the AMPK-SIRT1-PGC-1α pathway. Our studies investigate the effects of both natural and pharmacological metabolic alterations on learning and memory in rodents.

We assessed the differences in learning, short-term and long-term memory between Wistar rats treated with a regular dose of resveratrol (20 mg/kg; 5 d/week; p.o.), regular aerobic exercise (forced running; 1h/5d/week), both of these or neither. Animals were assessed on performance in the delayed non-matching-to-sample (DNMS) task over a 4 month protocol, with animals aged 14 months at the beginning. Training and testing in the DNMS task was carried out on the same days as exercise and resveratrol treatment.

We found that rats undergoing regular exercise and/or treated with resveratrol had improved performances compared to age-matched controls. The key improvements were found when assessed in the task with a 25-30 second delay. There was no difference between groups with no delay on the task. Furthermore, no evident side-effects arose from the administration of resveratrol for such a long epoch.

Our results indicate that regular resveratrol treatment and/or exercise help delay the hippocampal-dependent cognitive dysfunction associated with ageing. With no ill-effects observed, we have no evidence to suggest apprehension over regular administration of oral resveratrol with a long time-scale. The improved performance in this task associated with both natural and pharmacological metabolic enhancement highlight the potential use of these factors in delaying the neurological and metabolic deficits associated with ageing.

Disclosures: R.C. Hussey: None. **S.M. O'Mara:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.02/LLL1

Topic: F.02. Animal Cognition and Behavior

Support: Ellison Medical Foundation

NIH Grant P01-AG-09973

Title: Age-associated changes in cognition and inhibitory interneuron network integrity in diversity outbred mice

Authors: *M. KOH, A. SPIEGEL, M. GALLAGHER;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Failure of episodic memory, linked to hippocampal dysfunction, is a common but not inevitable feature of aging. Such age-related impairment can worsen to an extent greater than expected for a person's age, leading to a diagnosis of amnestic mild cognitive impairment (aMCI), a condition that increases risk for further progression to Alzheimer's dementia. Recent evidence from animal models and human clinical studies has demonstrated that diminished pattern separation, a computational function mediated by hippocampal circuits involving the dentate gyrus (DG), contributes to these age-related conditions of memory loss. Encoding deficits indicative of diminished pattern separation are associated with weakened input to CA3/DG originating from layer II entorhinal neurons and reduced integrity of somatostatin-positive (SOM+) hilar interneurons in memory-impaired aged rats. Here, we used the newly developed Diversity Outbred (DO) mouse to study individual differences in memory. The DO mouse population was designed to model the genetic diversity found in human populations, maximizing allelic variation throughout the genome while maintaining normal levels of hetereozygosity. The increased genetic diversity in the DO model produces high levels of phenotypic variation rendering it a more powerful system to investigate individual differences in behavior than common laboratory inbred rodent strains. We behaviorally characterized hippocampal-dependent memory performance in young (4-8 mo old) and aged (18-24 mo old) male DO mice in a water maze protocol. Young adult mice exhibited greater proficiency in learning to locate a hidden escape platform during training and stronger spatial memory for the escape location during probe trials compared to the overall performance of aged DO mice. A substantial number of older DO mice, however, performed on a par with the normative

performance of younger adults. Stereological quantification for SOM+ neurons showed that high-performing young and unimpaired aged DO mice had comparable numbers of SOM+ hilar interneurons, while aged mice that were impaired in the spatial task had significantly fewer such neurons. These data in the DO model extend findings that tie loss of SOM+ interneuron integrity to age-related memory impairment, as reported for individual differences in outbred Long-Evans rats, and in models of ApoE4 expression as mice exhibit augmentation of an aging effect on that specific interneuron population. The vulnerability SOM hilar interneurons, given their anatomical and functional attributes, could contribute to impoverished pattern separation as a basis for episodic memory failure in the aged brain.

Disclosures: M. Koh: None. **A. Spiegel:** None. **M. Gallagher:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.03/LLL2

Topic: F.02. Animal Cognition and Behavior

Support: PPG P01 AG 09973

Title: Voluntary exercise modifies interneuron protein expression in the dentate hilus

Authors: *A. M. SPIEGEL[1], S. SALAS-VEGA[1], A. M. STRANAHAN[2], M. GALLAGHER[1]; [1]Dept of Psych & Brain Sci., Johns Hopkins Univ., BALTIMORE, MD; [2]Physiol. Dept., Med. Col. of Georgia, Augusta, GA

Abstract: Voluntary exercise is associated with enhanced hippocampal dependent memory. Recent behavioral evidence indicates that an improved ability to disambiguate information with overlapping elements (i.e. pattern separation) contributes to this memory enhancement. Proficient pattern separation depends on sparse encoding within the dentate gyrus, with high divergence of cortical input onto granule cells and local circuits within the dentate contributing to this computational property. GABAergic interneurons in the dentate hilus play a critical role in sparse encoding in response to cortical input, as inhibition of these neurons increases the number of activated granule cells and impairs spatial memory. Little is known about the effects of voluntary exercise on the GABAergic network. Here we explored exercise-induced modifications on dentate hilar GABAergic interneurons. We found that voluntary exercise alters the phenotypic expression of markers for hilar interneuron populations in young, male Long Evans rats. Using immunohistochemistry with stereological quantification, the number of hilar interneurons expressing glutamic acid decarboxylase-67 (GAD67), Neuropeptide Y (NPY) and

Somatostatin (SOM) were quantified in sedentary rats and rats exposed to 30 days of voluntary wheel running. Runners reached a maximum daily running average of 3545m ± 857m, with asymptotic running performance from approximately day 12 onward. Running was associated with a 27% increase in the number of hilar neurons expressing GAD67. Voluntary exercise had a similar affect on hilar interneuron subgroups with NPY-expressing neurons exhibiting a 37% increase and SOM-positive neuron number increasing by 17%. Additionally, the total number of NPY and SOM-positive hilar neurons, which likely represent overlapping neuronal populations in the hilus, was highly correlated with GAD67-positive neuron number. Overall these data indicate that voluntary exercise is a strong modulator of the hilar GABAergic network. Given the functional importance of hilar interneurons in sparse encoding, their modulation could contribute to the beneficial effects of exercise on hippocampal dependent memory. The current work may also have relevance to the loss of GAD67 and SOM-positive hilar interneuron number previously reported in aged Long Evans rats with memory impairment. Considering that the reduction in GAD67 and SOM-positive neuron number occurs in the absence of frank degeneration, voluntary exercise may prove to be an effective treatment in the restoration of interneuron protein expression and memory function in aged impaired animals.

Disclosures: A.M. Spiegel: None. **S. Salas-Vega:** None. **A.M. Stranahan:** None. **M. Gallagher:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.04/LLL3

Topic: F.02. Animal Cognition and Behavior

Support: NIH RO1 Grant AG028084

State of Arizona

ADHS and the Arizona Alzheimer’s Disease Core

Title: Do anabolic steroids impact cognition? An evaluation of androstenedione's effects on cognition in young male rodents

Authors: *B. W. CAMP[1,2], R. HIROI[1,2], L. TORRES[1], L. KARBER[1], H. A. BIMONTE-NELSON[1,2];
[1]Psychology, Arizona State Univ., Tempe, AZ; [2]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: Following natural menopause, androstenedione becomes the main hormone secreted by the follicle-deplete ovaries. We have previously shown that higher serum levels of androstenedione are related to spatial memory impairment in female rats; this relationship was shown when androstenedione serum levels were of endogenous (Acosta et al., 2009a) or of exogenous (Camp et al., 2012), origin, and within high physiological levels. Thus, there is mounting evidence in support of androstenedione as a detriment to spatial memory in the female rat. We recognize that androstenedione has grown in popularity as a supplement taken by men, and might possibly impair spatial memory as we have noted in the female rodent model. Since androstenedione-induced memory effects in females cannot necessarily be generalized to effects in males, we applied this question to a male rodent model. This is a clinically relevant question as the use of androstenedione as a physical-performance enhancing steroid has greatly increased in the last 15 years, with little knowledge of the potential effects on brain function. In the current study, young gonadally intact male rats were given a daily injection of either vehicle or androstenedione, followed by a battery of cognitive tasks. Preliminary data suggest that effects in males are in congruence with those we have seen previously in females. Replication studies are underway, and the totality of work will be presented at the conference

Disclosures: B.W. Camp: None. **R. Hiroi:** None. **L. Torres:** None. **H.A. Bimonte-Nelson:** None. **L. Karber:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.05/LLL4

Topic: F.02. Animal Cognition and Behavior

Support: Arizona Alzheimer's Consortium

Title: Human navigation on a radial-arm maze: Strategy choice and performance outcome

Authors: *H. A. BIMONTE-NELSON[1], I. GRUNFELD[2], S. MENNENGA[1], B. CAMP[1], G. BREWER[1], L. BAXTER[2], M. MCBEATH[1];
[1]Arizona State Univ., TEMPE, AZ; [2]Dept. of Neuroimaging Res., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: This study examined differences in navigational strategy by translating between spatial reasoning models in rodent and human research. We built a 50-foot diameter, 11-arm walk-through Human Radial-Arm Maze (HRAM) to parallel the traditional Radial-Arm Maze (RAM) that has been a mainstay in rodent research to test spatial working memory. 157

participants performed the HRAM along with a battery of other cognitive tests. The HRAM required participants to traverse all 11 arms and recover play money hidden under mats at the end of each arm, with the goal to commit the fewest number of repeat entries into the arms (quantified as errors). Participants were not allowed to use simple ordered chaining strategies of entering adjacent arms, or of every second arm. Performance was evaluated by quantifying number of repeat arm entry errors, order of arm entries, and self-reported use of navigational strategies, such as utilizing external cues. The shape of the working memory load curve of arm-repetition errors over time was similar to rodent performance on the RAM, with more errors as choices progressed and load increased, while also consistent with a higher memory capacity in humans. We found two primary strategies: (1) Systematic procedural heuristics (repeatedly crossing over to opposing arms, or complex chaining such as traversing every third arm), and (2) Unsystematic, brute-force memorization (reliance on remembering intramaze features or global orientation cues). The 44% who used systematic heuristics made fewer errors than the 56% who relied on brute-force memory. 47% stated that they utilized external cues to solve the task; these participants were in both systematic heuristics and brute-force memory groups, therefore, use of extramaze cues did not correspond with that type of group membership. Additionally, we found an effect of learning/practice associated with repeat runs through the maze, and an effect of gender whereby males outperformed females. Sex differences in solving strategies are currently being evaluated. In conclusion, the HRAM resulted in two distinct navigational strategy categories: systematic heuristics and brute-force memory. Testing humans using methodology similar to that employed when evaluating rodent models provides a foundational tool to translate models of spatial cognition and reasoning across species. The HRAM shows promise as a functional translational instrument for testing memory and navigational principals commonly evaluated in rodents, and can be used to translate scientific questions between rodents and humans.

Disclosures: H.A. Bimonte-Nelson: None. **I. Grunfeld:** None. **S. Mennenga:** None. **B. Camp:** None. **G. Brewer:** None. **L. Baxter:** None. **M. McBeath:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.06/LLL5

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG028084

State of Arizona

ADHS and the Arizona Alzheimer's Disease Core

NIH Grant MH093145

Title: The impact of hormone therapy estrogens on memory, depressive-like, and anxiety-like behaviors: A comparison of conjugated equine estrogens and 17β-estradiol

Authors: *S. V. KOEBELE, R. HIROI, S. E. MENNENGA, L. T. HEWITT, P. K. MENDOZA, C. N. LAVERY, G. WEYRICH, L. F. KARBER, H. A. BIMONTE-NELSON; Psychology, Arizona State Univ., Tempe, AZ

Abstract: Aging and the menopausal transition have each been associated with cognitive impairment, and this is often co-morbid with anxiety as well as affective disorders such as depression. Low or changing levels of estrogens in women, as seen with peri- and post-menopause, have been related to cognitive, anxiety, and affective disorders. Benefits of 17β-estradiol (E2), the most potent naturally circulating estrogen in women and rats, on cognitive, anxiety-like, and depressive-like behaviors have been shown. Although conjugated equine estrogen (CEE) is the most commonly prescribed estrogen component of hormone therapy (HT) in menopausal women, there is a marked gap in knowledge regarding whether CEE affects cognition, anxiety-like, and depressive-like behaviors, and how these effects compare to E2. This is a clinically significant question since bio-identical HT utilizing E2 is gaining recognition as a viable option. For example, both estrogen types are being methodically evaluated and compared in the KEEPS clinical trial (Wharton et al., 2013). Here, using a rodent model, we evaluated the effects of CEE treatment on these behaviors, and compared them to those seen with E2 treatment. Female rats were ovariectomized, administered either vehicle, E2, or CEE, and tested on a battery of cognitive, anxiety-like, and depressive-like behaviors in the following order: Water Radial Arm Maze (spatial working and reference memory), Morris Water Maze (spatial reference memory), Delayed Match-to-Sample (spatial working and recent memory), Visible Platform (overall motor and visual ability), Open Field (anxiety-like behavior and overall locomotion), Elevated Plus Maze (anxiety-like behavior) and the Forced Swim Test (depressive-like behavior). Results showed that both E2 and CEE enhanced spatial working memory, corroborating previous reports in adult and middle-aged ovariectomized rats. Further, E2, but not CEE, decreased anxiety-like and depressive-like behaviors. Collectively, these results indicate that, while both estrogen treatments have functional consequences on behavior, the impact of CEE is not as broad as E2. In conclusion, there seems to be a dissociation between cognitive, anxiety and affective measures.

Disclosures: S.V. Koebele: None. **R. Hiroi:** None. **S.E. Mennenga:** None. **L.T. Hewitt:** None. **P.K. Mendoza:** None. **C.N. Lavery:** None. **G. Weyrich:** None. **L.F. Karber:** None. **H.A. Bimonte-Nelson:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.07/LLL6

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG028084

State of Arizona

ADHS and the Arizona Alzheimer's Disease Core Center

Title: Estradiol only improves performance when working memory load is very high: Alternative interpretation of poor performance on an easy task

Authors: *S. E. MENNENGA[1], J. E. GERSON[2], S. V. KOEBELE[1], L. T. HEWITT[1], A. S. JORDAN[1], A. A. MOUSA[1], H. A. BIMONTE-NELSON[1];
[1]Arizona State Univ., Tempe, AZ; [2]Univ. of Texas Med. Br., Galveston, TX

Abstract: 17β-Estradiol (E2) is the most potent naturally circulating estrogen, and is the most common estrogen evaluated in rodent learning and memory studies. There is evidence that E2 treatment induces cognitive benefit following ovariectomy (Ovx) in rats; however, this benefit seems to be limited to conditions involving a high working memory load. The Water Radial Arm Maze (WRAM) assesses spatial working and reference memory; there are several WRAM variations, including differences in the number of reinforcers subjects are required to locate within a testing session. The number of reinforcers animals are required to find within a session corresponds to the working memory load that the task requires. We hypothesize that the cognitive benefits of E2 treatment in young animals are specific to working memory tasks with a high memory load and, in fact, performance indicative of impaired memory may be seen in animals treated with E2 when the working memory demand is not high enough. Here, in two studies, the cognitive effects of tonic E2 administration were evaluated in young Ovx rats under different working memory loads. Study I assessed the cognitive effects of low, medium, and high doses of E2 using the WRAM with hidden platforms in 4 of 8 arms, requiring a lower working memory load. Study II assessed the cognitive effects of low and high doses of E2, with hidden platforms in 7 of the 8 arms, requiring a higher working memory load. For each study, animals were given 12 testing days, followed by a delay test. Results showed that, in young animals tested under the current parameters, E2-induced enhancements were only evident when requirements necessitated a high working memory load. There were no differences in reference memory errors regardless of the behavioral task, indicating that effects were specific to the working memory domain. Our data suggest that in young animals, the benefits of E2 treatment

become apparent only at high working memory loads, and that the benefits of E2 are not realized on tasks that do not sufficiently tax the working memory system. These results indicate task difficulty as a factor to be considered when evaluating the impact of female steroids on cognition.

Disclosures: S.E. Mennenga: None. **J.E. Gerson:** None. **S.V. Koebele:** None. **L.T. Hewitt:** None. **A.S. Jordan:** None. **A.A. Mousa:** None. **H.A. Bimonte-Nelson:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.08/LLL7

Topic: F.02. Animal Cognition and Behavior

Support: UNT Health Science Center Faculty Seed Grant

NIHP01 AG022550

Title: Curcumin supplementation improves certain aspects of cognition and alleviates inflammation, independent of adiposity

Authors: *M. SARKER, M. J. FORSTER, S. F. FRANKS, N. SUMIEN, F. FILIPETTO; UNT Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Midlife obesity has been recently associated with cognitive impairment that may be attributed to chronic, obesity-related inflammation and oxidative stress. The current study addressed the hypothesis that curcumin supplementation, by attenuating obesity and adiposity - related inflammation, would improve cognitive function in a midlife obesity animal model. Commonly used laboratory mice fed ad libitum are an analogue of weight gain in middle aged humans, since accumulating fat is more often the result of food intake exceeding energy expenditure and not solely because of a high fat diet. In this study, C57BL/6J male mice were maintained under ad libitum (AL) feeding until they reached peak weight at 15 months of age, as a model of inactivity-related weight gain. The mice were subsequently assigned in groups of 19 to (i) remain on AL, (ii) receive 30% caloric restriction (CR) or (iii) receive curcumin in their AL diet (1000 mg/kg diet, CURC) for 12 weeks. Mice underwent tail bleeds for the inflammatory markers, interleukin 6 (IL-6) and C-reactive protein (CRP) and, after 8 weeks of dietary treatment, spatial cognitive function was tested using a Morris water maze, followed by testing for cognitive flexibility using a discriminated avoidance, serial reversal task. Visceral and subcutaneous adipose tissue was collected after 12 weeks of the treatments. Mice maintained on

CR weighed significantly less than mice on the CURC and AL diets by the third week of treatment. Food intake of the CURC group was significantly higher than AL. Mice on CR and CURC diets took fewer trials than AL to reach criterion during the second reversal session of discriminated avoidance, suggesting that both conditions improved cognitive flexibility. On the other hand, there were no significant differences between the groups in their spatial cognitive performance. Mice maintained on CR had significantly less visceral and subcutaneous adipose tissue compared to mice on CURC and AL. Curcumin supplementation did not significantly impact IL-6 levels but it did reduce CRP relative to AL mice after 12 weeks of dietary treatment. The results suggest that, in a midlife obesity animal model, curcumin supplementation has positive effects on frontal cortical functions that may be linked to an anti-inflammatory action, but are independent of adiposity.

Disclosures: M. Sarker: None. **M.J. Forster:** None. **S.F. Franks:** None. **N. Sumien:** None. **F. Filipetto:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.09/LLL8

Topic: F.02. Animal Cognition and Behavior

Support: Abbott Laboratories project ZA69

Title: The effects of HMB on water maze performance in middle-aged and aged male and female rats

Authors: *D. G. KOUGIAS, W. A. KOSS, L. K. SHERRILL, E. R. HANKOSKY, L. R. HAMMERSLAG, J. M. GULLEY, J. M. JURASKA;
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Abstract: Beta-hydroxy-beta-methylbutyrate (HMB), a leucine metabolite commonly supplemented in elderly and clinical populations, has been shown to maintain muscle in catabolic states; however, its role in the aging brain remains unknown. In both healthy aging humans and in rat models, there are deficits in several modalities of cognitive functioning. Deficits in Morris water maze performance have been found in healthy aging rats in several labs. The current study explores the cognitive effects of short- and long-term (1 month and 7 month) HMB supplementation starting at 12 months of age in male and female Long-Evans hooded rats. At 11 months of age, female rats are ovariectomized to model the normal human female aging following menopause. Male rats undergo a sham surgery to control for the possible effects of

anesthesia exposure. HMB was administered in a sucrose solution (450 mg/kg twice daily). Short-term HMB supplemented male (n=12) and female (n=10) rats and short-term vehicle treated male (n=11) and female (n=9) rats were run on the water maze (4 trials/day for 4 days). There was a main effect of day ($p<.001$) in water maze performance, but no other effects were found. These results indicate no sex differences and no direct, immediate cognitive enhancing effects on water maze performance with short-term HMB supplementation in middle-aged rats. These data are the baseline from which the long-term HMB supplementation group will be compared to assess whether this nutrient mitigates cognitive decline in aging rats.

Disclosures: D.G. Kougias: None. **W.A. Koss:** None. **L.K. Sherrill:** None. **E.R. Hankosky:** None. **L.R. Hammerslag:** None. **J.M. Gulley:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Abbott Laboratories. **J.M. Juraska:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Abbott Laboratories.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.10/LLL9

Topic: F.02. Animal Cognition and Behavior

Support: NSERC 341673

CFI13176

Title: Effects of ghrelin knock-out and age on spatial learning, neurogenesis, and spine density in the dentate gryus of rats

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Abstract: Work has revealed a high density of ghrelin receptors distributed throughout the hippocampal CA1 and dentate gyrus (DG) subregions. Binding of ghrelin to these receptors is associated with elevated dendritic spine density in the CA1 region and increased neurogenesis in the DG. These ghrelin-induced neuronal changes are paralleled by enhanced learning and memory on a variety of hippocampal and non-hippocampal dependent tasks such as the water

maze after administration of ghrelin. While elevations in ghrelin appear to have consistent enhancing effects on spatial learning and hippocampal connectivity, the effect of decreasing ghrelin, either through antagonists or knock-out, has been less clear.
In the present experiment, we investigated how knock-out of the ghrelin receptor impacted spatial learning and memory as well as how ghrelin knock-out affected neurogenesis and spine density in the DG of the hippocampus. In this experiment, we used genetically modified rats, as opposed to mice, where previous work has been concentrated. To this end, we used both wild type (WT) and ghrelin receptor deficient (KO) Fawn Hooded Hypertensive (FHH) rats and tested them on two water maze (WM) tasks as well as an 8 arm radial arm maze task (8-RAM). To examine neurogenesis, we used double cortin immunohistochemistry to evaluate the number of immature neurons in the DG. Golgi- Cox impregnation was used to evaluate spine density in the DG. As aging is associated with decreased levels of ghrelin and has been shown to contribute to decreased neurogenesis and lead to impairments in learning and memory, we also tested theses WT and KO rats when they were aged.
Results showed there was no difference in acquisition on the water maze task between groups. Age was found to decrease learning and memory on the 8-RAM task, and this effect was exasperated in the aged KO rats. Both spine density and neurogenesis were decreased in the aged groups, with young WT rats having the highest spine density and levels of neurogenesis. Overall, results demonstrate a lack of an effect of ghrelin KO on water maze performance. An effect of ghrelin KO does appear to produce deficits in the performance of the 8-RAM food-motivated task, but only in aged rats. While in aging, there was an overall decrease in immature neurons and spine density, decreased neurogenesis and spine density was also evident in the young ghrelin KO rats. Data suggest that ghrelin KO disrupts hippocampal structural integrity that may manifest in non-spatial-related aspects of hippocampal function.

Disclosures: S.P. Cahill: None. **A. Abizaid:** None. **T. Hatchard:** None. **M.R. Holahan:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.11/LLL10

Topic: F.02. Animal Cognition and Behavior

Support: NIA

UWM RGI

Quincy Bioscience

Title: Aging reduces basal neuronal activation as measured by immediate early gene expression within medial prefrontal cortex

Authors: *M. SEHGAL[1], J. A. DETERT[3], T. S. BULA[1], J. R. MOYER, Jr.[1,2];
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Abstract: We have recently demonstrated that aging is accompanied by deficits in medial prefrontal cortex (mPFC)-dependent behaviors such as fear extinction starting in middle age *(Kaczorowski et al., 2012*). Our data also show that aging leads to region-specific changes in intrinsic excitability of mPFC neurons. Specifically, infralimbic (IL) neurons are less excitable and prelimbic (PL) neurons are more excitable in middle-aged and aged rodents. It is likely that these changes in excitability are reflected in reduced basal neuronal activity during normal aging. To measure region-specific changes in neuronal activation, we quantified basal immediate early gene (IEG) expression within mPFC. Basal IEG expression reflects ongoing memory consolidation (*Marrone et al., 2008*). Furthermore, the role of IEGs as markers of neuronal plasticity and their critical role in memory consolidation make them ideal for investigating early cognitive decline. The current study used western blots and immunohistochemistry to test the hypothesis that normal aging alters basal expression of Zif-268 and Arc in mPFC. Beginning in middle age, immunohistochemistry revealed a significant decline in the number of Zif-268 immunoreactive neurons within both IL ($p < 0.01$) and PL ($p < 0.05$). There were no significant differences in Zif-268 immunoreactivity between middle-aged and aged rats for either IL or PL. Western blots indicated a similar pattern of results for Zif-268 within mPFC. Zif-268 expression was reduced within IL for middle-aged and aged rats ($p < 0.05$). Within PL, Zif-268 expression was significantly reduced in both middle-aged ($p < 0.05$) and aged rats ($p < 0.08$). These data suggest that aging is associated with a decrease in both the number as well as the expression levels of Zif-268 protein. Basal expression of Arc followed a distinct pattern of aging-related changes. Western blots revealed that Arc expression was significantly reduced within IL of aged ($p < 0.05$) but not middle-aged rats relative to adult rats. Interestingly, Arc expression is unaltered in PL during normal aging. Since immunohistochemistry revealed a primarily dendritic location of Arc protein within mPFC, cell counts were not obtained. These data suggest that aging results in differential changes in the expression of Zif-268 and Arc within subregions of mPFC. Interestingly, aging-related changes in IEG expression are evident beginning in middle age, suggesting that they may contribute to our observed aging-related extinction deficits as well as deficits in other mPFC-dependent tasks.

Disclosures: M. Sehgal: None. **J.A. Detert:** None. **T.S. Bula:** None. **J.R. Moyer:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.12/LLL11

Topic: F.02. Animal Cognition and Behavior

Support: NIA

UW-Milwaukee RGI

Quincy Bioscience

Title: Early detection of region-specific changes in immediate early gene expression within hippocampus during normal aging

Authors: *J. R. MOYER, JR.[1,2], M. SEHGAL[1], J. A. DETERT[3], T. S. BULA[1]; [1]Psychology, [2]Biol. Sci., Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [3]Dept. of Anesthesiol., Weill Cornell Med. Col., New York City, NY

Abstract: Normal aging is accompanied by cognitive decline that differs from other aging-related pathological states like Alzheimer's disease. With an increasing proportion of the world population falling in an age group of 65 years and above, a preventive gerontological approach may be essential to ultimately improving the quality of life for the elderly. Especially relevant is identifying markers for early detection of cognitive decline. Although aging-related changes in hippocampal functioning have been well documented, relatively little is known about the age of onset of these changes, and the differential effects of aging on the dorsal (DH) and ventral (VH) subregions of hippocampus. Aging-related changes in the expression of memory permissive genes, e.g. immediate early genes (IEGs), can be especially useful markers in predicting early cognitive decline. Specifically, IEGs are markers of neuronal activity and are critical for long-term memory formation. Here we used Western blots and immunohistochemistry to quantify the expression of two IEGs, Zif-268 and Arc, within hippocampal subregions as a function of normal aging in male F344 rats. Preliminary studies suggest that aging resulted in a significant reduction in the expression of Arc in DH ($p < 0.01$) but not VH. Interestingly, these reductions were first observed in middle-aged animals and no further reductions in Arc expression were observed in aged DH. Immunohistochemistry revealed that basal Arc expression was localized to the dendritic compartment of DH as well as VH subregions. Analysis of Zif-268 revealed region-specific changes during aging. Western blots indicated that the total amount of Zif-268 protein did not change during aging in DH. However, immunohistochemical analyses revealed that in DH the number of Zif-268-ir neurons was significantly decreased in both CA1 ($p < 0.01$) and DG ($p < 0.05$) of both middle-aged and aged rats (no change was observed in CA3). Within VH, there was a trend toward a reduction in total Zif-268 protein expression in middle-aged and aged rats. Interestingly, in VH the number of Zif-268-ir neurons was significantly reduced, but only in DG ($p < 0.001$) of middle-aged and aged rats. These data indicate that the dorsal and ventral

subregions of hippocampus may undergo differential changes in gene expression during normal aging. Furthermore, within dorsal and ventral hippocampus, IEG expression changes were restricted to certain principal cell layers and not others. Given the role of IEG expression in neuronal plasticity, aging-related changes in IEG expression are likely markers for early onset of cognitive decline within these structures.

Disclosures: J.R. Moyer, Jr.: None. **M. Sehgal:** None. **J.A. Detert:** None. **T.S. Bula:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.13/LLL12

Topic: F.02. Animal Cognition and Behavior

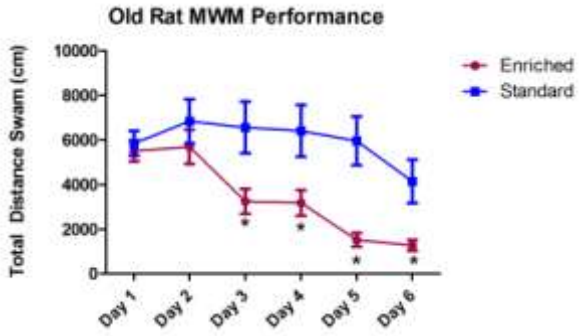
Support: National Science Foundation Graduate Research Fellowship Program Fellowship

Title: The molecular and cellular mechanisms behind environmental enrichment

Authors: *R. HULLINGER[1], K. O'RIORDAN[2], C. BURGER[2];
[2]neurology, [1]Univ. of Wisconsin- Madison, Madison, WI

Abstract: Behavioral and pharmacological treatments for cognitive impairments associated with the aging process are becoming a growing focus of concern as the elderly population continues to expand worldwide. Current evidence suggests that environmental enrichment can improve learning and memory, but the age at which enrichment can be effective and the length of enrichment necessary remains unclear. Furthermore, the impact of enrichment on synaptic plasticity and the molecular mechanisms behind enrichment are not completely understood. To address these unresolved issues, we have housed 2 month old, 12 month old, and 20 month old rats in enriched environments and conducted tests of learning and memory formation after 1 month and after 4 months of enrichment. Preliminary results demonstrate that 1 month of environmental enrichment improves performance on the Morris water maze, novel object recognition, and contextual fear conditioning for 20 month old rats, whereas 4 months of enrichment is necessary to improve performance on these tasks in 2 month old and 12 month old rats. Furthermore, we have found that environmental enrichment results in a robust expression of long term potentiation (LTP) within hippocampal area CA1 for all age groups, a phenomenon not observed in rats housed in standard environments. Currently we are investigating the molecular mechanism behind this cognitive and synaptic enhancement, with particular focus on changes in expression of genes known to be involved in LTP following a period of enrichment. The results of these studies will provide insight into the molecular basis of learning and memory

enhancement following environmental enrichment.
Representative data from the Morris water maze hidden platform training in 20 month old rats demonstrates that enriched rats perform significantly better than standard housed rats (Two- way anova, $p<.05$ on day 3, 4, 5, and 6).



Disclosures: R. Hullinger: None. **K. O'Riordan:** None. **C. Burger:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.14/LLL13

Topic: F.02. Animal Cognition and Behavior

Title: Identification of cognitive deficits and brain pathology in a mouse model of normal aging

Authors: *M. WEBER[1], T. WU[1], S. L. DOMINGUEZ[1], H. LIN[1], H. NGU[2], K. SCEARCE-LEVIE[1];
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Abstract: While age is the main risk factor for the development of sporadic Alzheimer's disease, age-related cognitive decline in rodents has been relatively less studied. This may in part be due to non-cognitive age-related changes like hearing or vision impairments, or changes in general locomotor activity that can confound cognitive tests in rodents. Here, we have compared 3, 11 and 23-month old female C57BL6/J mice in a battery of behavioral tests. Cognitive tests were complemented by tests of acoustic and visual functioning, and locomotor activity. Age-related impairments were detected in tests of spatial memory (Barnes maze), fear memory (cued fear conditioning), and associative learning (active avoidance). No age-related changes were observed in locomotor activity, and only minimal impairments were detected in visual placing and eye-blink tests. In contrast, physical responses to acoustic stimuli of varying intensities were

impaired in an age-dependent manner, indicating hearing impairments. In order to determine if the active avoidance deficits could be related to the hearing impairments, we assessed mice in an active avoidance variant in which light but no tones were presented as conditioned stimuli. Age-related deficits were again observed. Taken together, these data suggest that neither deficits in hearing, vision, nor locomotor activity are likely to account for the observed deficits in the cognitive measures. We are currently analyzing brain tissue from these mice for markers for inflammation and the effects of a novel environment challenge on c-fos expression in the hippocampus. The identification of robust deficits in cognition in aged mice provides an opportunity for a translational model of normal aging. Such a model would have value in screening for cognitive enhancers, and investigating the biological changes associated with aging-related cognitive decline.

Disclosures: M. Weber: A. Employment/Salary (full or part-time):; Genentech Inc. **T. Wu:** A. Employment/Salary (full or part-time):; Genentech Inc. **S.L. Dominguez:** A. Employment/Salary (full or part-time):; Genentech Inc. **H. Lin:** A. Employment/Salary (full or part-time):; Genentech Inc. **H. Ngu:** A. Employment/Salary (full or part-time):; Genentech Inc. **K. Scearce-Levie:** A. Employment/Salary (full or part-time):; Genentech Inc..

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.15/LLL14

Topic: F.02. Animal Cognition and Behavior

Support: RFBR (grant 13-04-00388)

Program of RAS “Fundamental Sciences to Medicine”

MRC UK

ARUK

Title: Study of the molecular mechanisms underlying cognitive dysfunction caused by prenatal hypoxia in rats

Authors: *I. A. ZHURAVIN[1], N. M. DUBROVSKAYA[1], S. A. PLESNEVA[1], A. J. TURNER[2], N. N. NALIVAEVA[1,2];
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Abstract: To elucidate the molecular mechanisms underlying disruption of cognitive functions caused by pathogenic factors (hypoxia, stress) during embryogenesis we have analysed neurophysiological characteristics of rats at various stages of postnatal ontogenesis as well as expression and activity of some enzymes in brain structures. Prenatal hypoxia (PH) in rats (E14, 3 hours, 7% O_2) led to an impairment of cognitive functions in later ontogenesis correlated with a decrease in the average density of labile synaptopodin-positive dendritic spines in the neocortex (Cx) (*Zhuravin et al, Dokl. Biol. Sci., 2009, 425, 123-5; 2011, 438, 145-8*). PH and ageing also shifted the metabolism of amyloid precursor protein (APP) and expression of a major amyloid-degrading enzyme neprilysin (NEP) towards the amyloidogenic pathway. The PH rats had decreased levels of AChE and NEP activity in the Cx and hippocampus (Hip). Moreover, ageing and administration of NEP or AChE inhibitors also resulted in deterioration of memory. Recently we demonstrated that the APP intracellular domain AICD up-regulates expression of NEP in a histone deacetylase (HDAC)-competitive mode (*Belyaev et al, EMBO Rep, 2009, 10, 94-100*). Injections to HP rats of an HDAC inhibitor valproic acid (VA) resulted in an enhanced NEP activity, increased number of labile spines and improved memory tested in the maze and by a novel object recognition test (*Dubrovskaya et al, Short-term memory: new research, Nova, 2012, 6, 155-173*). Injection of VA to rats also led to an increased binding of AICD and decreased binding of HDAC to the *NEP* promoter in rat Hip (*Nalivaeva et al, J Mol. Neurosci, 2012, 46, 569-577*). Treatment of rats with an antioxidant epigallocatechin gallate (capable of epigenetic regulation of gene expression) also resulted in an increased NEP activity, higher number of dentritic spines in the Hip and improved memory in PH rats. Thus, the impairment of cognitive functions caused by PH (as well as by ageing) involves a decrease in the activity of cholinergic and peptidergic systems accompanied by a reduction in brain synaptic plasticity. Regulation of these systems via epigenetic mechanisms can be a viable therapeutic strategy to improve cognitive functions impaired by prenatal stress.

Disclosures: I.A. Zhuravin: None. **N.M. Dubrovskaya:** None. **S.A. Plesneva:** None. **A.J. Turner:** None. **N.N. Nalivaeva:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.16/LLL15

Topic: F.02. Animal Cognition and Behavior

Support: ANR grant 2010 MALZ 001-02

Polish fellowhip (POKL.04.01.01-00-051/10-00 for J.P.).

CNRS

Title: NMDA receptor subcellular location and memory functions are altered in the APP/PS1 mouse model of Alzheimer's disease

Authors: S. HADZIBEGOVIC[1], J. PORęBSKA[2], Y. CHO[3], N. MACREZ[1], B. BONTEMPI[1], *O. NICOLE[1];
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Abstract: The cognitive impairments in Alzheimer's disease (AD) are related to degenerative synaptic changes produced by the presence of soluble Aβ oligomers (Aβos) in vulnerable brain regions such as the hippocampus. At the molecular level, Aβos bind preferentially to the postsynaptic density of neuronal excitatory synapses, where the post-synaptic protein-95 (PSD-95) organizes NMDA receptor composition and location as well as its downstream signaling. We speculate that that in AD, early alterations in scaffolding protein expression following synaptic accumulation of Aβos may modify the NMDAR subcellular trafficking, which would in turn alter the plasticity-related events triggered by learning and memory processes. To test this hypothesis, we first analyzed the differential expression of total synaptic proteins in the hippocampus of APP/PS1 transgenic (n=13; 9-10 month old) and wild-type (Wt) mice (n=13). As previously described in patients with mild cognitive impairment (Sultana et al., 2010), we also report a decrease of PSD-95 expression in APP/PS1 mice. Moreover, by using two hippocampal-dependent memory tasks (spatial object recognition and Y-maze), we found that the cognitive performance of APP/PS1 mice was impaired and correlated with the hippocampal expression of PSD-95. Since PSD-95 organizes NMDA receptor trafficking, we next examined the expression and distribution of GluN2 in hippocampal neurons. We provide evidence that the total expression of GluN2A was reduced in APP/PS1 mice whereas the total expression of GluN2B was unaffected. To evaluate the distribution of GluN2B between synaptic and extrasynaptic compartments, we performed a fractionation of synaptosomes and foundthat the reduced expression of PSD-95 was associated to a mislocalization of the GluN2B subunit. Indeed, the synaptic level of GluN2B was lower in APP/PS1 compared to Wt mice whereas the extrasynaptic pools of GluN2B receptors was higher in APP/PS1 compared to Wt mice. Moreover, the cognitive performance of APP/PS1 mice was inversely correlated with the level of extrasynaptic GluN2B receptors. Together, these results suggest that the post-synaptic accumulation of Aβos destabilizes synaptic organization and increases the activation of extrasynaptic NR2B-containing receptors, a reorganization which translates into impaired memory functions.

Disclosures: S. Hadzibegovic: None. **O. Nicole:** None. **J. Porębska:** None. **Y. Cho:** None. **N. Macrez:** None. **B. Bontempi:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.17/LLL16

Topic: F.02. Animal Cognition and Behavior

Support: KRF-2010-0023880

Title: Protective effects of P2X7 receptor deletion on aging-related cognitive status

Authors: *W. CHO[1], S.-R. LEE[1], J.-C. PARK[1], J.-R. LEE[2], J.-S. HAN[1];
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Abstract: P2X7 receptor (P2X7R) is an ATP-gated cation channel predominantly expressed on microglial cells in central nervous system. The P2X7 receptor can modulate cell growth, proliferation, apoptosis, and cell death. Recent studies have shown that inhibition of P2X7R ameliorates memory impairments induced by ischemia. The present experiment was conducted to examine effects of P2X7 receptor deletion on age-related cognitive status. Cognitive status of mice in 4 groups (5 month-old young P2X7R knock-out (KO) group, 11-14 month-old middle P2X7R KO group, two aged-matched wild type group (WT)) was assessed using Morris water maze task. No differences were observed between young WT and young KO mice in the spatial memory. However, middle-aged P2X7R KO mice showed better performance than age-matched WT mice. And levels of inhibitory phosphorylation at Serine-9 were higher in the hippocampus of young KO, middle-aged KO, and middle-aged WT mice compared to those of young WT mice. These results suggest that the deletion of P2X7R have a positive influence on aging-related alternations. The regulation of P2X7R might a potential therapeutic strategy for treatment of aging-related cognitive deficits. Supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2010-0023880) to Jung-Soo Han
Key Words: Aging, P2X7 receptor, memory, hippocampus, pGSK-3β

Disclosures: W. Cho: None. **S. Lee:** None. **J. Park:** None. **J. Lee:** None. **J. Han:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.18/LLL17

Topic: F.02. Animal Cognition and Behavior

Title: The sigma 1 receptor selective ligand ls-1-137 attenuates scopolamine induced impairment in learning and memory

Authors: *M. MALIK[1], C. BARAJAS[1], N. SUMIEN[1], R. MACH[2], R. LUEDTKE[1]; [1]Pharmacol. and Neurosc, Univ. of North Texas Hlth. Sc Ctr., Fort Worth, TX; [2]Div. of Radiological Sci., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Cognitive deficit is observed in patients with Alzheimer's Disease, Parkinson's Disease, traumatic brain injury and stroke. These types of cognitive deficits are often due to alterations in cholinergic signaling. Currently available therapeutic drugs provide only symptomatic relief and generally become ineffective as a neurodegenerative disorder progresses. Therefore, novel therapeutic agents will be needed to retard and/or arrest the progressive loss of memory forming cells. In this study, C57BL/6J mice injected with scopolamine (1mg/kg) were used as our experimental model to evaluate the ability of a sigma 1 receptor selective compound to improve the cognitive deficits associated with muscarinic antagonist administration. Scopolamine-induced memory impairment provides a relatively rapid and reversible phenotypic screening paradigm for cognition enhancement drug discovery. In this study, we evaluated LS-1-137, which exhibits high affinity (3.9nM) and sigma 1 versus sigma 2 receptor binding selectivity. It also binds with low affinity at D2-like (D2, D3 and D4) dopamine receptors. The cognitive state of the mice was evaluated using the water maze, active avoidance test and novel object recognition tests. Scopolamine treated animals exhibit impaired learning and memory in the water maze and the active avoidance test. C57BL/6J mice pre-treated with test drug at a dose of 3mg or 10mg/kg attenuated the scopolamine-induced cognitive deficit. Therefore, LS-1-137 represents a novel candidate cognitive enhancer for the treatment of cognitive deficits.

Disclosures: M. Malik: None. **C. Barajas:** None. **N. Sumien:** None. **R. Mach:** None. **R. Luedtke:** None.

Poster

579. **Animal Cognition: Learning and Memory - Aging I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 579.19/LLL18

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG038266

NIH Grant AG029421

NIH Grant AG020572

McKnight Brain Institute

Title: Selective deterioration of excitatory synapses in the aged dentate gyrus: Comparisons among hippocampal glutamatergic, GABAergic and cholinergic synapses

Authors: *J. A. MCQUAIL[1,2], J. P. HIBBLE[3], J. L. BIZON[1], B. D. SHUGOLL[3], M. M. NICOLLE[2,4,3];
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Abstract: Age-related memory decline is associated with alterations to hippocampal synapses. Although various systems change with age, few studies have simultaneously investigated the integrity of multiple neurochemical-specific synaptic markers within the same animal. The current study measured changes in levels of vesicular transporter proteins that package acetylcholine (VAChT), GABA (VGAT) and glutamate (VGluT1) for synaptic release using tissue sections prepared from young (6 mo; n=9) and aged (24-25 mo; n=25) Fisher × Brown Norway F1 hybrid rats that were previously characterized for place learning ability in the Morris water maze. Behavioral analysis revealed that aged rats exhibited a broad range of individual differences in spatial learning performance, including subjects with obvious impairment (aged-impaired), but also a subset of aged rats that were behaviorally similar to young (aged-unimpaired). Histological sections were immunofluorescently labeled for VAChT, VGAT and VGluT1 proteins and scanned on a Typhoon FLA 9500 imaging system at a final resolution of 10 µm. Expression of all 3 markers was measured in the dorsal hippocampus using Image J software. This analysis revealed a significant reduction in VGluT1 expression in the hippocampus of aged rats compared to young, but this reduction was not related to spatial learning performance. Subregional analyses localized these age-related reductions in VGluT1 to the molecular layer of the dentate gyrus but notably, VGluT1 expression was unchanged by age in CA3 and CA1. Hippocampal expression of both VAChT and VGAT did not differ with respect to age or cognitive status. These data indicate that there is a specific, age-dependent deterioration in the biochemical composition of excitatory terminals in the molecular layer of the dentate gyrus. This loss is both system- and subregion-specific, as proteins that serve equivalent functions at cholinergic and GABAergic terminals were not affected by age nor was VGluT1 expression attenuated in CA3 and CA1 subfields. Ongoing analyses combining confocal microscopy and stereological methods are focused on determining whether observed differences

are due to biochemical reductions within synapses or reduced number of biochemically-defined synapses.

Disclosures: J.A. McQuail: None. **J.L. Bizon:** None. **J.P. Hibble:** None. **B.D. Shugoll:** None. **M.M. Nicolle:** None.

580**Poster**

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.01/LLL19

Topic: F.02. Animal Cognition and Behavior

Support: NAT-VIEP-BUAP 2012-2013.

Title: The chronic treatment with L-DOPA impairs spatial working memory and increasing the nitrosative stress in the striatum and prefrontal cortex in rats with a 6-OHDA lesion

Authors: *G. RAMÍREZ GARCÍA, V. PALAFOX-SÁNCHEZ, I. D. LIMÓN PEREZ DE LEÓN;
Lab. De Neurofarmacología, Puebla, Mexico

Abstract: L-3,4-dihydroxyphenylalanine (L-DOPA) is administered over long term to improve motor functions in Parkinson‘s disease (PD) patients. Unfortunately, many motor complications such as on-off phenomenon, wearing-off, and dyskinesia arise over the years. There is evidence that metabolism of dopamine formed by L-DOPA generates free radicals such as nitric oxide, which may cause damage by nitrosative stress in the nigro-striatum-cortical pathway. This neuronal circuit has been implicated in cognitive processes such as spatial working memory (SWM). This memory type refers to the cognitive ability to update the information required to resolve a spatial task when contextual conditions change from one trial to the next.
In our work, we evaluated the effects of a L-DOPA chronic administration on SWM and nitrosative stress in SNpc, striatum and cortex prefrontal (PFC) in 6-hydroxydopamine (6-OHDA) lesioned rats. Control group received SSI and another group received orally L-DOPA/Carbidopa (100 mg/kg) for 20 days. After treatment the SMW task in water maze was done and then all animals were sacrificed in order to do the biochemical determinations. The nitrite levels were measured using the Griess method. The 3-nitrotyrosine (3-NT), induced nitric oxide synthase (iNOS) immunoreactivity was evaluated by immunofluorescence in the SNpc, striatum and PFC.
Our results showed that the L-DOPA treatment increases the deficit in SWM task moreover increased nitrites levels (15 %, 75 % and 58 %), the proteins nitration (74 %, 31 % and 118 %)

and up-regulation of the iNOS expression (132 %, 177 % and 155 %) in the SNpc, striatum and PFC respectively. These facts suggest that the L-DOPA induces stress nitrosative which could play a key role in impairment of synaptic communication involves in cognitive process such as SWM in rats with dopaminergic nigral lesion. Furthermore, in early stages of the PD the patients show deficit in SWM, these results propose that nitrosative stress is a probably toxicity mechanism caused by chronic administration L-DOPA in this PD patients.

Disclosures: G. Ramírez García: None. **V. Palafox-Sánchez:** None. **I.D. Limón Perez de León:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.02/LLL20

Topic: F.02. Animal Cognition and Behavior

Support: ANR Grant Sero6cognet

Ligue contre le cancer

Title: The neurofibromin directly interacts with the 5-HT6 serotonin receptor and regulates its function

Authors: *W. DERAREDJ[1], L. COBRET[1], F. GODIN[1], S. CHAUMONT[2], P. MARIN[2], H. BÉNÉDETTI[1], S. MORISSET[1];
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Abstract: The serotonin 6 (5-HT6) receptor is almost exclusively localized in the CNS, predominantly in regions involved in the regulation of cognitive processes such as the cerebral cortex, the hippocampus and the striatum. Correspondingly, it has become an increasingly promising target for the treatment of cognitive deficits associated with various CNS disorders including Alzheimer's disease and schizophrenia. Although 5-HT6 receptors are known to signal through Gs-adenylyl cyclase, coupling pathways and mechanisms controlling their activity remain poorly explored. To address these issues, the team of P Marin has recently used proteomic approaches to identify novel signalling proteins associated with the receptor. These studies revealed that 5-HT6 receptor is coupled to the mTor pathway. Furthermore they demonstrated the interaction of the receptor with Neurofibromin (Nf1), which is known to control synaptic plasticity, learning and memory formation. Neurofibromin is a large protein

(280kDa) encoded by the NF1 gene. It is ubiquitously expressed at low levels with highest expression levels in nervous system cells (neurons, Scwhann cells, and oligodendrocytes) and in leucocytes. Mutations of the NF1 gene are responsible of the most common genetic diseases, type 1 neurofibromatosis (NF1), which affects 1 in 3500 individuals. The phenotype of NF1 is highly variable, with benign (neurofibromas) or malignant peripheral nerve sheath tumors, CNS tumors (gliomas, astrocytomas) and cognitive deficits (40% of NF1 patients).
In this study, we characterized biochemically 5HT6 receptor/Nf1 interaction, which, to our knowledge, constitutes the first connection between Nf1 and a GPCR. Co-immunoprecipitation experiments performed from HEK-293 cells coexpressing various Flag-tagged domains of Nf1 and HA-5-HT6 receptor indicated that three different domains of Nf1, the SecPH, the GAP and the C-terminal domains, are capable of interacting with the receptor. Moreover, using Bioluminescence Resonance Energy Transfer (BRET), we demonstrated that the SecPH domain interacts with the 5-HT6 receptor directly and with the highest affinity compared with the other domains. Our data also demonstrated that 5-HT6 receptors can constitutively associate with Nf1 independently of its agonist stimulation. The implications of these interactions on 5-HT6 downstream signalling pathway (cAMP production, mTor pathway activation) are being analysed. In conclusion, 5-HT6 receptor identified as a new partner of neurofibromin constitutes an interesting target to understand signalling pathways involved in cognitive deficits of NF1 patients.

Disclosures: W. Deraredj: None. **L. Cobret:** None. **F. Godin:** None. **S. Chaumont:** None. **P. Marin:** None. **H. Bénédetti:** None. **S. Morisset:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.03/LLL21

Topic: F.02. Animal Cognition and Behavior

Support: PROINNOVA-CONACYT 2011, 2013

Palsgaard Industri de Mexico, S. de R.L de CV

Title: Effect of consumption of T2 dietary supplement in the short and long term memory and spine dentritic formation in mice

Authors: *M. I. TORRES-FLORES[1], C. FÉRNANDEZ-AGUILAR[3], S. GORDILLO-HIGAREDA[2], A. RAMÍREZ-RAMOS[3], E. PORTILLO-NAVARRO[3], M. RAMÍREZ-FLORES[3,2], R. HARO VALENCIA[3], M. APODACA-ARAGÓN[3], E. SÁNCHEZ[3], O.

GARCÍA[3,2];
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Abstract: Down syndrome (DS) or trisomy 21, is the most frequent genetic cause of mental retardation. Several nutritional therapies have been implemented to reduce the cognitive deficits in SD patients. Recently we developed a food supplement named T-2. The consumption of supplement T-2 in DS patients improves attention and sleep quality. However, the cellular mechanisms involved in these improvements, and the effect it could have consumption supplement T-2 on other behaviors are unknown. In this work we explored whether: 1) administration of supplement T-2 in wild type mice has an effect on tasks associated with learning and memory processes and 2) whether the consumption supplement T-2 induce changes in dendritic spines of hippocampus. Our results show that two months after consuming supplement T-2, mice improve the performance on Novel Object Recognition (NOR) task, a model associated to short- term recognition memory; however, had no effect on spatial memory evaluated through aquatic "Y" maze. In addition we observed that consumption supplement T-2 increase the density dendritic spines. These results suggest that the improvements observed by use of T-2 supplement in mice and DS patients could be associated to an increase in the number of dendritic spines.

Disclosures: M.I. Torres-Flores: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Palsgaard Industri de Mexico, S. de R.L de CV. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Palsgaard Industri de Mexico, S. de R.L de CV. **C. Férnandez-Aguilar:** A. Employment/Salary (full or part-time):; Palsgaard Industri de Mexico, S. de R.L de CV. **S. Gordillo-Higareda:** None. **A. Ramírez-Ramos:** A. Employment/Salary (full or part-time):; Palsgaard Industri de Mexico, S. de R.L de CV. **E. Portillo-Navarro:** A. Employment/Salary (full or part-time):; Palsgaard Industri de Mexico, S. de R.L de CV. **M. Ramírez-Flores:** A. Employment/Salary (full or part-time):; Palsgaard Industri de Mexico, S. de R.L de CV. **R. Haro Valencia:** A. Employment/Salary (full or part-time):; Palsgaard Industri de Mexico, S. de R.L de CV. **M. Apodaca-Aragón:** A. Employment/Salary (full or part-time):; Palsgaard Industri de Mexico, S. de R.L de CV. **E. Sánchez:** A. Employment/Salary (full or part-time):; Palsgaard Industri de Mexico, S. de R.L de CV. **O. García:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Palsgaard Industri de Mexico, S. de R.L de CV.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.04/LLL22

Topic: F.02. Animal Cognition and Behavior

Support: DHHS/NIH/NIMH/IRP

Title: Dopaminergic and cholinergic mediation of within-session concurrent discrimination learning

Authors: A. WYLIE[1], J. SEIDEMAN[3], D. YU[2], C. BLACKWELL[1], M. MISHKIN[1], *J. N. TURCHI[1];
[1]LN, [2]NIF, NIMH, NIH, Bethesda, MD; [3]Ctr. for Neural Sci., NYU, New York, NY

Abstract: Prior work has shown that monkeys can learn a set of visual discriminations presented concurrently only once daily on successive days (24-h ITI task) based on habit formation, served by a visuo-striatal circuit that is independent of the visuo-rhinal circuits serving one-trial recognition memory. Likewise, it has been reported that systemic antagonism of dopaminergic receptors via haloperidol impairs performance on the 24-h ITI task, but fails to have an impact on recognition memory. Conversely, systemic administration of the muscarinic receptor antagonist scopolamine causes deficits in one-trial visual recognition but has only a negligible impact on concurrent visual discrimination learning. In a recent report, monkeys were trained on a short-ITI version of concurrent visual discrimination learning. Stimulus pairs were repeated not only across daily sessions but also several times within each session (with roughly 4-min ITIs). The baseline discrimination learning rates were substantially reduced in this short-ITI version, from ~11 trials/pair to criterion on the 24-h ITI task to ~5 trials/pair on the 4-min ITI task, and systemic injections of either haloperidol or scopolamine impaired this more rapid learning (~16 trials/ pair and 12 trials/ pair, respectively). In the current study, we compared the effects of local, bilateral, microinfusions of the dopamine D2-selective antagonist eticlopride (6.36 mM) into the tail of caudate nucleus (2 μl/site) and ventral putamen (3 μl/site), combined with microinfusions of scopolamine (20 mM) into the perirhinal cortex (3 μl/site), with the effects of infusing equivalent volumes of saline into these same targets. Preliminary evidence (n=2) indicates that while learning rates were similarly rapid following local saline infusions to these regions (~3 trials/pair), the combined local drug administration impeded learning (~14 trials/ pair). Separate drug infusions within these different regions will enable us to elucidate the visuo-striatal and visuo-rhinal circuits that operate in tandem during within-session concurrent discrimination learning.

Disclosures: A. Wylie: None. **J. Seideman:** None. **C. Blackwell:** None. **M. Mishkin:** None. **J.N. Turchi:** None. **D. Yu:** None.

Poster

580. Animal Learning and Memory: Pharmacology I

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.05/LLL23

Topic: F.02. Animal Cognition and Behavior

Support: NIA Grant R21AG042745

Virginia Center on Aging

Title: FAAH inhibition improves memory performance in APP/PS1 transgenic mice

Authors: *L. E. WISE, B. C. RIEDEL, A. H. LICHTMAN;
Pharmacol & Toxicol, Virginia Commonwealth Univ., RICHMOND, VA

Abstract: The present research evaluated a novel potential treatment for Alzheimer's disease. Specifically, we treated APP/PS1 transgenic mice chronically with an inhibitor of fatty acid amide hydrolase (FAAH), the enzyme that is responsible for the breakdown of the naturally occurring marijuana-like brain chemical (i.e., endogenous cannabinoid) anandamide. This FAAH inhibitor, PF3845, produces up to ten fold increases in brain anandamide levels, elicits anti-inflammatory effects, and is devoid of marijuana's psychomimetic effects. We proposed that PF3845 would have anti-inflammatory and neuroprotective actions and consequently have beneficial effects on memory performance in the water maze in APP/PS1 transgenic mice, an in vivo mouse model of Alzheimer's disease. APP/PS1 transgenic mice and their non-transgenic littermates were administered 10 mg/kg PF3845 or vehicle for eight weeks (once daily for five days each week) starting at five months of age. Memory performance was evaluated in the water maze starting when the mice were seven months of age. We found that the APP/PS1 transgenic mice had memory impairments in the fixed platform water maze task as compared to non-transgenic mice. Repeated treatment with PF3845 improved memory performance in the water maze in the APP/PS1 transgenic mice, but not in the non-transgenic mice. Additionally, we also evaluated whether acute treatment with vehicle or 10 mg/kg PF3845 (i.e., drug administration only before water maze testing) improves memory function in APP/PS1 transgenic mice. In contrast to repeated treatment, acute treatment with PF3845 did not improve water maze performance in APP/PS1 transgenic mice. Our results indicate that PF3845 improves memory performance in APP/PS1 transgenic mice suggesting that FAAH inhibition may have beneficial effects on cognition in Alzheimer's disease. Future studies will determine whether repeated treatment with PF3845 also decreases neuropathological markers associated with Alzheimer's disease in APP/PS1 transgenic mice.

This research was supported by the Virginia Center on Aging (AHL and LEW) and the National Institute on Aging [R21AG042745 (LEW)].

Disclosures: L.E. Wise: None. **A.H. Lichtman:** None. **B.C. Riedel:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.06/LLL24

Topic: F.02. Animal Cognition and Behavior

Title: Are pre-training habituation sessions a valid method in the study of learning and memory in rats and mice?

Authors: *R. M. ABUHAMDAH[1], P. CHAZOT[1], S. ABUHAMDAH[2], A. ENNACEUR[3]; [1]Durham Univ., Durham, United Kingdom; [2]Univ. of Jordan, Amman, Jordan; [3]Univ. of Sunderland, Sunderland, United Kingdom

Abstract: In most studies of learning and memory, animals are introduced to a behavioral test with the implicit assumption that pre-training habituation sessions are sufficient to equalize the level of emotionality between groups. However, the number of these sessions is defined a-priori, and varies widely between laboratories. Depending on the number and duration of the habituation sessions, anxiety from novelty and unfamiliarity may be retained over the training sessions, and prevent the acquisition of a cognitive task, particularly in animals that had a brain lesion or that received a chronic drug treatment before introduction to the test apparatus.
In a series of experiments, we exposed mice to a 3D radial-maze without preliminary habituation sessions. In this maze, mice need to cross a bridge radiating from a central platform to reach onto an arm. We used different strains of mice (Balb/c, C57BL/6J and CD-1), aged male and female CD-1 mice, and C57BL/6J mice treated with dizocilpine.
When exposed for the first time to this maze, mice display fear and anxiety. Anxious mice do move beyond the bridges. In our experiments, all mice made numerous entries onto the bridges and took longer time to cross onto the arms of the maze. C57, CD-1 and Balb/c mice made respectively 8 arm choices in the second, third and fifth sessions. Though Balb/c appeared the most anxious strain, they performed better than C57 and CD-1 in subsequent training sessions. In a second experiment, C57 received ip injection of 0.1 mg/kg of MK-801 before each test session. This led to significant increase in the number of crossings onto the bridges, and a decrease in the number of crossings onto the arms. In the third experiment, we used young and adult CD-1 of both sexes. They all required 3 sessions to make 8 arm choices. Male adult made significantly

more bridge entries and more arm repeats compared to the other groups.
The present studies indicate that the number of habituation sessions cannot be determined a-priori. Emotional responses to novelty and test environment vary between strains and could be affected by an experimental manipulation such as lesions, drugs or genetic manipulation.

Disclosures: R.M. Abuhamdah: None. **P. Chazot:** None. **S. Abuhamdah:** None. **A. ennaceur:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.07/LLL25

Topic: F.02. Animal Cognition and Behavior

Support: NIH T32 ES07051

Title: Effects of methamphetamine on allocentric learning and memory in rats

Authors: *A. GUTIERREZ[1], R. M. AMOS-KROOHS[2], M. T. WILLIAMS[3], C. V. VORHEES[3];
[1]Neurosci. Grad. Program, [2]Mol. and Developmental Biol., Univ. of Cincinnati, Col. of Med., Cincinnati, OH; [3]Div. of Neurol., Cincinnati Children's Res. Fndn., Cincinnati, OH

Abstract: Housing animals in cages with reduced bedding has been shown to increase stress-related glucocorticoid release. Excessive stress adversely affects learning and memory (L&M) whereas moderate stress can have the opposite effect. Neurotoxic doses of methamphetamine (MA) impair some types of L&M but effects on allocentric L&M have been ambiguous. The present study investigated the effects of standard vs. low bedding (barren cage) housing in combination with neurotoxic MA on Morris Water Maze (MWM) L&M using a larger than normal pool size to increase task difficulty (244 cm diameter). After acclimation, male Sprague-Dawley rats were placed in cages with a paper towel only (Barren) or standard bedding (Std.) 3 weeks prior to treatment. Half of the animals in each housing condition received 10 mg/kg MA x 4 at 2 h intervals and half received saline (s.c.) on the same schedule. Testing began two weeks later. Testing began with rats receiving 4 trials in a 244 cm straight swimming channel to acclimate them to swimming and assess swim speed. The MWM consisted of 23 days given in 4 phases. The first 3 phases were hidden platform trials each being 6 days (4 trials/day) with a single probe trial on day-7 with no platform. Platform positions and sizes varied with each phase: acquisition (SW, 10 cm), reversal (NE, 7 cm), and shift (NW, 5 cm). The 4th phase was cued for 2 days (4 trials/day; 10 cm platform) with a ball mounted above the platform to mark its location.

There were no effects on swim speed in the straight channel or on cued trials. On hidden platform trials, MA-treated groups (regardless of housing) were significantly impaired in finding the platform on all 3 phases. There was no effect of MA on acquisition-probe, but MA-treated animals had longer average distance to the platform site on reversal and shift-probe trials. Barren housing only affected acquisition. Barren-housed rats, regardless of treatment, reached the platform more efficiently than Std. housed rats. These data suggest that a greater pool size to platform ratio is able to reveal clear spatial L&M deficits following MA-induced neurotoxicity. (Supported by NIH T32 ES07051)

Disclosures: A. Gutierrez: None. **R.M. Amos-Kroohs:** None. **M.T. Williams:** None. **C.V. Vorhees:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.08/LLL26

Topic: F.02. Animal Cognition and Behavior

Title: Discovery of Lu AF58801, a novel, selective and brain penetrant positive allosteric modulator of alpha-7 nicotinic acetylcholine receptors: Attenuation of subchronic phencyclidine (PCP)-induced cognitive deficits in rats following oral administration

Authors: *J. F. BASTLUND[1], C. BUNDGAARD[1], K. DEKERMENDJIAN[1], R. L. PAPKE[3], J. P. REDROBE[1], K. FREDERIKSEN[2], J. ESKILDSEN[2];
[1]H. Lundbeck A/S, Valby, Denmark; [2]H. Lundbeck A/S, valby, Denmark; [3]Dept. of Pharmacol., Univ. of Florida Col. of Med., Gainesville, FL

Abstract: α7 nicotinic acetylcholine receptors (nAChR*α*7) are expressed in brain areas involved in cognitive processing such as the hippocampus, thalamic nuclei, and frontal cortex, and decreased expression has been reported in diseases associated with memory impairment, including schizophrenia and Alzheimer's disease. To this end, compounds like EVP6124 and TC5619, acting as agonists of the nAChR*α*7's, have recently been shown to alleviate cognitive disturbances associated with schizophrenia and alzheimer's disease in small clinical phaseII studies. In contrast to agonists, nAChR*α*7 positive allosteric modulators (PAM) can reinforce endogenous cholinergic transmission without directly stimulating the target receptor. Accordingly, nAChR*α*7 selective PAMs have emerged as potential superior drug target class. We describe a novel nAChR*α*7 PAM, Lu AF58801, which is a potent, orally available, brain

penetrant PAM of the α7 nicotinic acetylcholine receptor, showing efficacy in a novel object recognition task in rats treated subchronically with phencyclidine (PCP).

Disclosures: **J.F. Bastlund:** A. Employment/Salary (full or part-time):; H.Lundbeck A/S. **C. Bundgaard:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **K. Dekermendjian:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **R.L. Papke:** F. Consulting Fees (e.g., advisory boards); Consulting for H. Lundbeck A/S. **J.P. Redrobe:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **K. Frederiksen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **J. Eskildsen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.09/LLL27

Topic: F.02. Animal Cognition and Behavior

Support: Psi Chi Undergraduate Research Grant

Title: The effect of cocaine on delay discounting in the spontaneously hypertensive rat

Authors: M. CLASEN[1], S. SEQUEIRA[1], J. J. O'MALLEY[1], A. SHEMERY[1], S. MCVAY[1,2], D. HOLT[1], *J. DYCHE[1];
[1]Psychology, James Madison Univ., Harrisonburg, VA; [2]St. Louis Univ., St. Louis, MO

Abstract: Cocaine intoxication is characterized by maladaptive behavioral or psychological changes including affective blunting, changes in sociability, anxiety and impaired judgment. Perhaps the most controversial and adverse side effects of long-term cocaine use are increased impulsivity which is seen in human and animal models (Moeller et al., 2001; Simon, Mendez, & Setlow, 2007). Impulsivity is behaviorally defined as picking a smaller, immediate reward (impulsive choice) over a larger, later reward (self-control choice) and is neurobiologically linked to dopamine receptors in the dorsal and ventral striatum. In animals, the spontaneously hypertensive rat (SHR) is one of the most widely used models of ADHD. The SHR exhibits the major behavioral symptoms of ADHD as well as having face validity and construct validity (Sagvolden, 2000). The literature indicates that cocaine seeking behavior is also linked to connectivity in the dorsal and ventral striatum (Belin & Everitt, 2008). Therefore, the present study examined the effects of acute cocaine administration on impulsive behavior measured by an adjusting-amount delay discounting procedure. Implementing a pre-test and post-test experimental design, baseline rates of discounting were measured. Four Sprague Dawley and

four SHR rats were sensitized to cocaine HCl for 14 days at a dosage of 30 mg/kg through intraperitoneal injections during sensitization. Following cocaine sensitization was an intermediate period for 28 days where the rats were housed in home cages. Through the use of an adjusting amount procedure used by Green et al., (2004) we calculated indifference points for each rat. An indifference point is defined as the point which the animal has the same preference for the immediate, adjusting reward and the delayed, standard reward. Results demonstrated the SHR were more impulsive than the Sprague Dawley and the animals had differential impulsivity after cocaine sensitization. This indicates that short-term use of cocaine may have effects on impulsive choice well after cocaine administration has terminated. Additionally, the plasticity of the striatum is implicated and future studies will examine the functions of metabatropic dopamine receptors in delay discounting.

Disclosures: M. Clasen: None. **S. Sequeira:** None. **J.J. O'Malley:** None. **A. Shemery:** None. **D. Holt:** None. **J. Dyche:** None. **S. McVay:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.10/LLL28

Topic: F.02. Animal Cognition and Behavior

Title: The role of NMDA receptors in the consolidation of habit memory

Authors: *K.-C. LEONG, M. G. PACKARD;
Psychology, Texas A&M Univ., College Station, TX

Abstract: Extensive evidence indicates that the dorsolateral striatum selectively mediates the acquisition of stimulus-response habit memory. However, relatively few studies have addressed the role of glutamate receptors within the dorsolateral striatum in consolidation of stimulus-response habits. The present experiment was designed to examine the potential role of the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor in the consolidation of dorsolateral striatal-dependent habit memory. Adult male Long-Evans rats received training (6 trials per day for 4 days) in a dorsal-striatal-dependent "forced-response" water plus-maze task, in which rats were trained to swim from varying start points (north, south) and to make the same body turn response (e.g. turn right) at the choice point of the plus-maze in order to reach a hidden escape platform. Immediately following training, rats received posttraining infusions of AP5 (2 µg), an NMDA antagonist, directly into the dorsolateral striatum on days 1 to 3 of training. Posttraining administration of AP5 impaired memory consolidation in the response learning task relative to

control animals that received posttraining vehicle infusions. These results suggest that NMDA receptors within the dorsolateral striatum are critical for the consolidation of stimulus-response habit memories.

Disclosures: K. Leong: None. **M.G. Packard:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.11/LLL29

Topic: F.02. Animal Cognition and Behavior

Title: The separate or concurrent effects of methylphenidate and alcohol on acquisition and retention of the Morris Water Maze in adolescent rats

Authors: M. CREECH, L. SCOTT, K. LIVESAY, *L. BAKNER;
Psychology, Linfield Col., MCMINNVILLE, OR

Abstract: Alcohol's (A) capacity to impair learning and memory has been well documented in the Morris Water Maze (MWM) but few studies have examined methylphenidate's (MPH) impact on MWM performance (Haidun et al., 2010; Zeise et al., 2007). Even fewer studies have evaluated concurrent administration of these two drugs in adolescent rats (see Markwiese, et al., 1998). This project used a rat model of adolescent drug use to examine individual effects of MPH and A, as well as polypharmacy interactions between MPH and A, on MWM spatial acquisition and retention.
32, adolescent (P30), male Long-Evans hooded rats were used. Subjects were assigned to one of 4 conditions based on drug administered prior to 6 consecutive acquisition sessions. Animals received 2 i.p. injections prior to each session. The methylphenidate group (MPH+S) received 2 mg/kg MPH and 1 ml/kg saline solution (S), the alcohol group (A+S) received 2 g/kg ethanol and S, the methylphenidate and alcohol group (MPH+A) received both MPH and A, and the saline control group (S+S) received S injections. MPH was administered 50 mins prior to each session and A administered 20 mins prior to each session. Each session consisted of 4 trials and rats swam from one of four start locations (N,E,S,W) to a submerged platform in the NE quadrant. Trial duration was 60 seconds and rats remained on the platform for 10 secs. Performances were video recorded, and latency and swim accuracy scored. Whishaw Corridors established a direct swim path from start location to platform and an error was recorded when swim paths exited the corridor. On day 7, the submerged platform was removed and a single, 60 sec retention test was conducted with no drug administered prior to test. Amount of time spent

swimming in the NE quadrant was analyzed to assess retention.
Acquisition. Both dependent measures, latency and swim accuracy, yielded similar outcomes. Factorial ANOVAs and post hoc tests showed improvement across training sessions for all groups. Importantly, the MPH+A group was impaired relative to all other conditions, and the S+S group performed better than the A group. No significant differences were observed between S+S and MPH+S groups.
Retention. A One-way ANOVA of swim time in the NE quadrant revealed longer swim times for the S+S group compared to the A+S group, and longer swim times for the MPH+S group compared to the A+S group. No other significant differences were observed.
While all groups improved performance during acquisition, methylphenidate + alcohol compromised spatial learning and alcohol alone impaired learning relative to controls. Interestingly, measures of retention indicated only alcohol diminished spatial memory in adolescent rats.

Disclosures: M. Creech: None. **L. Scott:** None. **K. Livesay:** None. **L. Bakner:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.12/LLL30

Topic: F.02. Animal Cognition and Behavior

Title: Effects of intrastriatal naloxone infusion on spatial navigation performance in the rat: Implications for the treatment of obsessive compulsive disorder

Authors: J. PISCOPELLO, N. CHABAN, *B. D. DEVAN;
Psychology, Towson Univ., TOWSON, MD

Abstract: The present study investigated the possible role of mu-opiate receptors within the dorsal striatum in the spatial navigation performance of rats trained in the water maze. Intrastriatal guide cannula were stereotaxically implanted to target the anteromedial part of the caudate-putamen (CPu) complex and following a 2-3 week recovery period, subjects were trained to find a hidden platform at a fixed location in the water maze for 16 days (4 trials/day). On day 10, rats were given a passive placement test to assess checking behavior. On day 17 (retraining), subjects were infused bilaterally with the mu-opiate receptor antagonist naloxone (15 µg/side) or vehicle control (0.9% NaCl) and received four escape trials with the platform moved to a new location. A 60 sec place competition test was given the following day. Intrastriatal naloxone infusion slowed swim speed but did not alter escape performance during

retraining. The naloxone group showed shorter path lengths and entered the 'old' radial quadrant less than controls on the competition test. The present findings provide evidence for the involvement of the mu-opiate receptor-rich striatal patch compartment in the higher-order habit control of associative spatial processing (Devan, Hong & McDonald, 2011, Neurobiol Learn Mem, 96:95-120). Implications for the treatment of OCD symptoms such as compulsive checking are discussed.

Disclosures: J. Piscopello: None. **N. Chaban:** None. **B.D. Devan:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.13/LLL31

Topic: F.02. Animal Cognition and Behavior

Title: Motor impairment and behavior alteration tested in open field in 21 day-old male mice postnatally treated with midazolam

Authors: *A. MARQUEZ-OROZCO[1], I. JIMENEZ-ESTRADA[2], G. DE LA FUENTE-JUAREZ[1], S. SANTIAGO-LOPEZ[1], J. JOYA-VENEGAS[1], A. FORTANEL.FONSECA[1], A. FORTANEL.FONSECA[1], M. MARQUEZ-OROZCO[1];
[1]Dept Embryol, Univ. of Mexico (UNAM), Mexico 04510 DF, Mexico; [2]Physiology, Biophysics and Neurosciences, CINVESTAV-IPN, Mexico DF, Mexico

Abstract: Midazolam (MDZ) is a hypnotic benzodiazepine that rapidly induces tranquility and deep sleep during brief surgical procedures performed with local anesthetic. The drug is easily transported into the brain after intravenous infusion. MDZ induces an anterograde amnesia that creates an illusion of anesthesia in some patients. Intravenous administration of MDZ has been widely used in pediatric patients for conscious sedation in procedures such as lumbar punctures and bone marrow aspiration.
MDZ administered postnatally alters the development of both neuronal and Purkinje cells in cerebral and cerebellar cortices. The purpose of this work was investigated if 21-day-old male mice postnatally treated with MDZ develop motor impartment and behavior alterations tested in open field.
Two neonatal ICR strain male mice groups were injected daily sc from day 6 to 9 postnatal. The first group (MDZ) was treated with single daily MDZ doses (1.0 mg/kg/bw/sc) and the second group (C) with saline solution. The pups (10 MDZ and 10 C), were tested in an open field 50x50x20 cm for 5 min at 21-days of age. The open-field behavior was video-recorded. In a

screen the open field was divided in 9 equal squares and the number of squares visited by each mouse was registered during 5min. The distance (cm) and velocity (cm/s) were analyzed using a computerized digital system of the Department of Physiology, Biophysics and Neurosciences, CINVESTAV-IPN. The male mice of the MDZ group displayed a significantly higher exploratory behavior than the C mice group. The mean of distance run by MDZ male mice group, the velocity and the number of squares visited were statistically significant higher in the MDZ group mice than in the C group mice. The alterations observed may be attributed to delayed neuronal differentiation and cell neurodegeneration produced in cerebral and cerebellar cortices induced by MDZ exposition from 6 to 9 days old male mice.
Drugs that potentiate GABAA receptors such as MDZ and diazepam can trigger widespread apoptotic neurodegeneration. In the case of MDZ it has been previously shown that doses sufficient to maintain surgical anesthesia for 6 hr in 7-day-old rats cause widespread apoptotic neurodegeneration in the developing brain and persistent memory/learning impairment. We found similar results, which may be explained by the histological changes in the cerebral and cerebellar cortices induced by postnatal exposure to MDZ that we found in our trial.

Disclosures: A. Marquez-Orozco: None. **I. Jimenez-Estrada:** None. **G. De la Fuente-Juarez:** None. **S. Santiago-Lopez:** None. **J. Joya-Venegas:** None. **A. Fortanel.Fonseca:** None. **A. Fortanel.Fonseca:** None. **M. Marquez-Orozco:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.14/LLL32

Topic: F.02. Animal Cognition and Behavior

Support: Clark Foundation

Title: High-energy diet alters intrinsic excitability in young hippocampal CA1 neurons: Gender-dependent responses to insulin

Authors: *E. UNDERWOOD, L. T. THOMPSON;
The Univ. of Texas At Dallas, Richardson, TX

Abstract: Alarming increases in dietary fat intake and subsequent rises in obesity/insulin resistance/type II diabetes necessitate a focus on the effects of diet on normal physiological brain function and on cognitive performance. Rats trained in a passive-avoidance task exhibit enhanced memory retention 24 hr or more later when intrahippocampal insulin is administered immediately post-acquisition (Babri et al., 2007). Successful consolidation of many different

learning tasks (e.g. inhibitory avoidance training or trace eye-blink conditioning) reduce Ca2+ dependent afterhyperpolarizations (AHPs) of hippocampal CA1 pyramidal neurons (Farmer & Thompson, 2012). Since successful learning of a task is accompanied by a reduction of AHPs (i.e. an increase in intrinsic excitability) and insulin enhances memory retention, we investigated the effects of a chronic high-energy diet, which can alter basal insulin, on measures of CA1 hippocampal neuron intrinsic excitability which regulate information transfer to hippocampal efferents: effects of diet and of insulin on post-burst afterhyperpolarizations.
Male and female young adult Long-Evans rats (4-6 mo) were fed from weaning either a control diet (14% fat, 64.8% carbohydrate, and 21.2% protein) or a high-energy diet (57.6% fat, 26.8% carbohydrate, and 15.6% protein) prior to brain slice preparation. The experimental high-energy diet was augmented with slow-digesting casein protein and medium-chain triglyceride polyunsaturated coconut oil to achieve the desired ratios. After slice preparation, in vitro current clamp recordings were made to assess post-burst AHPs, accommodation, and passive membrane properties. After baseline recordings, brain slices were perfused with insulin (0, 6, 12.5, 25, or 50 nM) and recordings were repeated.
Young control female CA1 neurons had smaller peak AHP amplitudes (~4.0 mV), both reduced medium AHPs (mAHPs) and reduced slow AHPs (sAHPs), and reduced accommodation (fired more spikes to a sustained depolarization), while on all these measures male control neurons were less intrinsically excitable. After 2 mo on chronic high energy diet, this gender-dependent profile was not only reversed, but intrinsic excitability of CA1 neurons from both genders was significantly reduced. Neurons from females on the high-energy diet had significantly larger mAHPs and sAHPs of longer duration and area than those from males, which were still significantly enhanced by the diet compared to controls. These neurophysiological changes, occurring within a relatively short time frame, could have significant cognitive consequences, which will be assessed in the next phase of our studies.

Disclosures: E. Underwood: None. **L.T. Thompson:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.15/LLL33

Topic: F.02. Animal Cognition and Behavior

Title: α7 nicotinic acetylcholine receptor (α7 nAChR) antagonists as potential cognition enhancers

Authors: *N. P. VAN GOETHEM[1], L. WENNOGLE[2], H. STEINBUSCH[1], J. PRICKAERTS[1]; [1]Psychiatry & Neuropsychology, Maastricht Univ., Maastricht, Netherlands; [2]Intra-Cellular Therapies, Inc., New York, NY

Abstract: α7 nicotinic acetylcholine receptors (α7 nAChRs) are ligand-gated ion channels expressed primarily in the brain. These receptors have been implicated in modulating many cognitive functions like attention, episodic memory and learning. α7 nAChRs are located both pre- and postsynaptically and modulate glutamate, GABA and dopamine. Furthermore, α7 nAChRs are directly involved in hippocampal long-term potentiation (LTP), the putative cellular mechanism underlying learning and memory. Activation of α7 nAChRs through selective, partial or full agonists and/or modulators, has been shown to improve cognitive function in both animal and human studies. The main cognitive improvement with these compounds relate to memory, in accordance with the high level of expression of α7 nAChRs in the frontal-cortex and hippocampus. Hence, α7 nAChR agonists/modulators may be attractive drug candidates to improve cognition in Alzheimer's disease (AD) and schizophrenia patients. The objective of the current study was to investigate the cognition enhancing properties of low dose administration of selective α7 nAChR antagonists in rats as low doses of MLA have sporadically been reported to improve cognition in animals. Furthermore, MLA has been shown to facilitate LTP induction in hippocampal region CA1 in rats..Memory performance was assessed with the object recognition task (ORT). The compounds used for these studies were methyllycaconitine (MLA) and Compound 7i. MLA is a norditerpenoid alkaloid produced by many species of Delphinium (larkspurs) and is often used because of its ability to potently antagonize the α7 nAChRs Compound 7i is a relatively new selective α7 nAChR antagonist.. In the current study, it was found that both Compound 7i and MLA improved memory of rats in the ORT natural forgetting (i.e. 24h retention interval) paradigm (optimal dose range: Compound 7i; 0.1-1.0 mg/kg, MLA; 0.003-0.1 mg/kg, i.p.). Moreover, it was found that a dose that was too high to improve memory in the natural forgetting paradigm (Compound 7i; 3.0 mg/kg, MLA; 1.0 mg/kg, i.p.), was also sufficient to induce a memory deficit in the 1 h retention interval ORT, an interval that normally leads to good memory performance of rats. Among other possibilities, one explanation for these findings could be that α7 nAChR antagonists promote α7 nAChR resensitization. This hypothesis is currently investigated. In summary, while the main focus of the α7 nAChR as a target for cognition enhancement lies on agonists and positive modulators, the antagonists of these receptors might also prove to be a valuable tool for cognition enhancement in AD or schizophrenia.

Disclosures: N.P. Van Goethem: None. **L. Wennogle:** None. **H. Steinbusch:** None. **J. Prickaerts:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.16/LLL34

Topic: F.02. Animal Cognition and Behavior

Support: 5P20RR016463

8P20GM103423

Title: Prenatal choline supplementation prevents behavioral and neural impairments of adult MK-801 exposure in male rats

Authors: *C. A. NICKERSON[1], A. L. BROWN[2], M. J. GLENN[2];
[2]Psychology, [1]Colby Col., Waterville, ME

Abstract: Choline is essential to the development and function of the central nervous system. Supplemental levels, especially in development, protect against a variety of neural insults. The goal of the present study was to investigate whether prenatal choline supplementation protects against behavioral deficits and neural dysfunction precipitated by dizocilpine (MK-801) given in adulthood. MK-801 is an NMDA receptor antagonist frequently used in rodent models of psychological disorders. At low doses, it impairs cognition and at high doses it impairs motor function and degenerates axons. In this study, timed-pregnant female rats were fed either a control synthetic diet (AIN67A with 1.1 g/kg choline chloride; STD) or a choline-supplemented diet (AIN67A with 5 g/kg choline chloride; SUP). At birth, pups were cross-fostered and reared in mixed litters. As adults, 39 male rats were assigned to a saline or MK-801 condition, resulting in 4 groups: STD-SAL (n=9); STD-MK (n=10); SUP-SAL (n=10); SUP-MK (n=10). To assess memory, we used the novelty preference task of object recognition. After a 5-min study phase, rats received saline or MK-801 (0.2 mg/kg i.p.) injections and memory was assessed 3 hr later. MK-801 significantly impaired memory in STD rats; this effect was prevented in SUP rats. Two weeks later, rats were given a larger MK-801 injection (3 mg/kg i.p.) and motor function was evaluated for 3 hrs post-injection. MK-801 significantly increased locomotion, stereotypy, and ataxia over the course of the observation period in STD rats; these effects were significantly attenuated in SUP rats. Three days later, rats were sacrificed under isoflurane anesthesia and decapitated. Extracted brains were post-fixed and sectioned on a vibratome. A series of 30-µm frontal sections were retained for Fluoro-Jade B staining to mark degenerating axons. STD-MK rats displayed non-significant increases in marked axons compared to all other groups--a finding consistent with protection in SUP rats. A series of 60-µm hippocampal sections were retained for doublecortin immunohistochemistry to mark new, immature neurons. In this case, STD-MK rats displayed significantly fewer new neurons compared to the other groups. Taken together, these findings provide overwhelming evidence that SUP rats were protected against the toxic effects of

MK-801 on behavior and choline may exert this protection by slowing injury to axons and declines in neural plasticity. In particular, the capacity for choline to mitigate the motor defects induced by MK-801 was a novel and compelling result. This research contributes to the growing body of evidence supporting the robust neuroprotective capacity of choline.

Disclosures: C.A. Nickerson: None. **M.J. Glenn:** None. **A.L. Brown:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.17/LLL35

Topic: F.02. Animal Cognition and Behavior

Support: Pharmacog IMI Grant 115009

Title: The effect of donepezil and memantine on impaired spatial memory performances in a non-human primate, the grey mouse lemur

Authors: A. RAHMAN[1], F. PIFFERI[1], Y. LAMBERTY[2], E. SCHENKER[3], *M. SPEDDING[4], R. BORDET[5], J. C. RICHARDSON[6], F. AUJARD[1];
[1]UMR 7179, CNRS/MNHN, Brunoy, France; [2]Neurosci. Therapeut. Area, UCB Pharma s.a., Braine l'Alleud, Belgium; [3]Insitut de Recherches Servier, Croissy-sur-Seine, France; [4]Scientific Cooperation & Business Develop., LES LABORATOIRES SERVIER, Suresnes Cedex, France; [5]Dept. de Pharmacologie M´edicale,, Universit´e Lille Nord de France, Lille, France; [6]R &D China, GlaxoSmithKline, Stevenage, Herts, United Kingdom

Abstract: Background: Clinical trials have shown the evidance of symptomatic benefits of acetylcholinesterase inhibitors such as donepezil (DPZ) and an N-methyl-D-aspartate (NMDA) receptor antagonist, memantine (MEM) in patients with Alzheimer's disease (AD). However, only 40% of patients respond positively to this treatment. The grey mouse lemur, a non-human primate, is one of the very few animals that spontaneously develop most of the AD-related neuropathologies. Here we tested the efficacy of DPZ and MEM on impaired spatial memory performance following sleep deprivation (SD) used as non pharmacological challenge to induce transient cognitive impairment. This study is part of a work performed on mouse lemur to validate this model for future drug testing programs in AD.
Methods: Thirty three adult male mouse lemurs aged between 2 to 3 years were used. Cognitive impairment was induced by 8h of SD and spatial memory performance was assessed in a radial arm maze. In a 2-day protocol, mouse lemurs underwent training and testing session on day 1. On day 2, animals were submitted to 8h SD and retested afterwards. The sleep deprived group

(SD) underwent the protocol without any intervention. The DPZ-treated group received intraperitoneal 0.1mg/kg (DPZ-0.1) and 1mg/kg (DPZ-1) and MEM-treated group 0.1mg/kg (MEM-0.1) and 1mg/kg (MEM-1) 3h before the cognitive function test. The saline treated group (Sal) received only saline. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU on the Care, Welfare and Treatment of Animals.
Results: The "SD" group showed an increase of the number of errors (NE) and of the latency to reach the goal box during testing, which were significantly higher than that of training phase (NE=3.45± 0.93 vs 6.14±1.72, p=0.045; latency 293.2±43.8 vs 465.5±49.1, p=0.042). This SD-induced deficit was not observed in the "DPZ-0.1" (p= 0.127) and "DPZ-1" (p=0.246) group. The "Sal" group showed a significantly higher NE than that of training phase (4.83± 1.10 vs 2.44± 0.36, p=0.013). The effect of MEM is yet to be completed.
Conclusion: The present results indicate that donepezil was able to prevent the cognitive impairment induced by SD in the mouse lemur. This further support the utility of this model, in particular for evaluating novel AD therapeutics.
The research leading to these results was conducted as part of the PharmaCog consortium funded by the European Community's Seventh Framework Programme for the Innovative Medicine Initiative under Grant Agreement n°115009. For further information please refer to www.pharmacog.org

Disclosures: A. Rahman: None. **M. Spedding:** None. **F. Pifferi:** None. **Y. Lamberty:** None. **E. Schenker:** None. **R. Bordet:** None. **J.C. Richardson:** None. **F. Aujard:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.18/LLL36

Topic: F.02. Animal Cognition and Behavior

Title: Posttraining peripheral administration or intra-dorsolateral striatum injection of the cannabinoid 1 (CB1) receptor agonist WIN 55, 212-2 impairs the consolidation of habit memory

Authors: *J. GOODMAN, M. G. PACKARD;
Psychology, Texas A&M Univ., College Station, TX

Abstract: Converging evidence indicates that the cannabinergic system plays a role in modulating memory processes. Previous work from our laboratory employing response learning in a water plus-maze task indicates that posttraining peripheral administration of a cannabinoid

agonist impairs the consolidation of habit memory. In the present study, we examined whether this impairing effect of peripheral cannabinoids can be replicated using a visible platform water maze task and whether posttraining infusions of a cannabinoid agonist into the dorsolateral striatum (a brain region well-known for its role in consolidation of habit memory) also produces an impairment in this habit memory task. Adult male Long-Evans rats were trained for 1 day (for 8 trials) in a cued platform water maze task in which rats were released from different start points and in order to escape had to find a cued platform which was moved to various spatial locations across trials. Immediately following training, rats received an i.p. injection of the CB1 receptor agonist WIN 55, 212-2 (1mg/kg or 3mg/kg) or a vehicle solution. In a separate study using the same water maze task, rats received posttraining bilateral infusions into the dorsolateral striatum of WIN 55, 212-2 (100ng/.5μL or 200ng/.5μL) or vehicle solution. 24 hours later rats were given two probe trials, and the latency to reach the platform on these two trials served as an index of memory. Relative to the vehicle-treated controls, peripheral WIN 55,212-2 at the 3mg/kg dose significantly impaired memory ($p < .05$). Bilateral intra-striatal infusions of WIN 55, 212-2 at 200ng/.5μL also produced a significant impairment of memory ($p< .05$). Taken together, the results indicate that peripheral or intra-striatal administration of a CB1 receptor agonist impairs consolidation of dorsolateral striatal-dependent memory. We suggest that pharmacological agents that target the cannabinergic system may prove useful in the treatment of some human psychopathologies, in particular those characterized by strong habit-like behavioral features.

Disclosures: J. Goodman: None. **M.G. Packard:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.19/LLL37

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA029252

Title: Methylphenidate enhances olfactory discrimination reversal learning in rats

Authors: S. E. MAGGIO[1], *J. GALIZIO[2];
[1]Psychology, [2]Univ. North Carolina, Wilmington, NC

Abstract: Recent work has shown conflicting results in the effects of methylphenidate (MPD) on reversal learning of spatial tasks, but there is little relevant research on non-spatial reversal learning. In the current study, the effects of MPD on reversal learning with olfactory stimuli in

six male Sprague-Dawley rats were examined. Using an automated olfactometer, subjects were trained in a two-odor discrimination task and its reversal. Each subject received testing on a multiple within-session discrimination reversals in baseline, and after saline, 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg doses of MPD. The 1.0 mg/kg dose of MPD increased the rate of reversal learning in one of the six subjects. The 3.0 mg/kg dose of MPD produced an increase in reversal learning above saline levels in five subjects. Impairments in reversal learning were observed in most rats at the 10.0 mg/kg MPD dose, but this dose also impaired accuracy on a well-learned discrimination, and seems most likely to involve a general performance deficit. In sum, the 3.0 mg/kg dose of MPD produced fairly consistent enhancement of reversal learning with olfactory stimuli using this repeated discrimination reversal procedure.
Research supported by grant DA029252

Disclosures: S.E. Maggio: None. **J. Galizio:** None.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.20/LLL38

Topic: F.02. Animal Cognition and Behavior

Support: Translational Methamphetamine AIDS Research Center NIDA grant P50 DA26306

Title: Interactions of methamphetamine and HIV gp120 expression on discrimination learning in the mouse

Authors: *J. P. KESBY, A. MARKOU, S. SEMENOVA;
Psychiatry, Univ. of California San Diego, La Jolla, CA

Abstract: HIV-infected patients show deficits in learning and executive function. Methamphetamine (METH) abuse is common among HIV-infected individuals. However, whether METH abuse exacerbates learning deficits in patients with HIV remains unclear. The HIV/gp120 protein induces neuropathology in mice similar to HIV-induced pathology in humans. We investigated the separate and combined effects of METH exposure and expression of the HIV/gp120 protein on cognitive function in adult male transgenic (gp120TG) and control mice (nonTG).
Mice were exposed to an escalating METH binge regimen. Two months later, mice were tested in the attentional set-shifting task (ASST) assessing discrimination and reversal learning. The ASST included simple discrimination, compound discrimination, compound reversal, intradimensional shift and intradimensional reversal stages. Two exemplars (odor or platform

texture) were counter-balanced between test groups. The percentage of mice that failed to complete each stage (i.e. failure rate) and the number of trials to criterion (six consecutive correct responses) were analyzed.
The platform exemplar was significantly more difficult for the mice to discriminate than the odor exemplar as reflected by significantly more trials required to complete stages with platform discrimination and greater failure rates compared to odor discrimination stages. There was no effect of gp120 expression or METH exposure on the failure rate in the odor-based ASST. However, the failure rate of gp120TG mice in the platform-based ASST was significantly greater compared with nonTG mice. METH exposure exacerbated the gp120-induced failure rates in the platform-based ASST. Independent of METH exposure or the exemplar used, gp120TG mice required significantly more trials than nonTG mice to complete the discrimination, but not the reversal, stages suggesting that increased failure rates were due to impaired discrimination learning.
These results demonstrate that gp120 expression impairs discrimination learning in mice and this effect is exacerbated by METH exposure. The interaction of METH exposure and gp120 expression were only revealed under experimental conditions with increased task difficulty. Our findings in gp120TG mice suggest that learning deficits in humans with HIV infection may be partially due to the effects of the gp120 protein and deficits may be exacerbated with METH abuse. Thus, understanding the role of HIV-related proteins in HIV-induced learning deficits may allow for improved treatment of specific aspects of cognitive performance in HIV-infected patients.

Disclosures: J.P. Kesby: A. Employment/Salary (full or part-time):; University of California San Diego. **A. Markou:** A. Employment/Salary (full or part-time):; University of California San Diego. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Bristol-Myers-Squibb. **S. Semenova:** A. Employment/Salary (full or part-time):; University of California San Diego. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Translational Methamphetamine AIDS Research Center NIDA grant P50 DA26306.

Poster

580. **Animal Learning and Memory: Pharmacology I**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 580.21/LLL39

Topic: F.02. Animal Cognition and Behavior

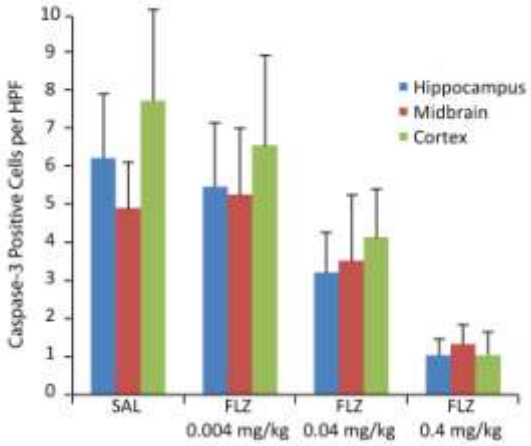
Support: Atlanta Research and Education Foundation

Departmental Resources

Title: The benzodiazepine antagonist, flumazenil, mitigates the post-anesthesia effects of the inhaled anesthetic, isoflurane

Authors: J. A. FIDLER[1,2], B. L. RAYMOND[2], S. C. BURKE[3], S. R. BABER[1], C. KARLAPALEM[2], *P. S. GARCIA[1,2];
[1]Anesthesiol., VA Med. Ctr., Decatur, GA; [2]Anesthesiol., Emory Univ. Sch. of Med., Atlanta, GA; [3]Anesthesiol., Univ. of California San Francisco, San Francisco, CA

Abstract: Emergence from general anesthesia involves activation of several monoaminergic arousal pathways in the brain stem. Recently, pharmacologic inhibition of GABA activity using flumazenil has been shown to improve vigilance in hypersomnic subjects. In this study, the authors tested the hypothesis that flumazenil - widely considered a rescue drug for benzodiazepine toxicity - improves recovery from anesthesia. Using adult rats anesthetized for 30 minutes with isoflurane, a GABA-enhancing inhaled anesthetic, the authors tested the effect of flumazenil on time to recover the righting reflex (RR), as well as its effect on physiologic parameters, general activity, and memory in the post-anesthesia state. A histological examination of anesthetic-induced neuro-apoptosis was also performed. Flumazenil (0.4 mg/kg) decreased mean time to RR by an average of 63 seconds (mean ± sd: flumazenil, 179.2 ± 46.7 s; saline, 242.2 ± 83.3 s). Memory testing revealed an improvement in novel object recognition (NOR) with the administration of flumazenil. Flumazenil had little effect on general activity or physiologic and respiratory factors under general anesthesia. Histologic examination revealed a dose-dependent decrease in caspase-3 positive cells in the hippocampus, midbrain, and cortex in animals administered flumazenil upon cessation of isoflurane anesthesia (Figure). The authors conclude that flumazenil actively promotes emergence from isoflurane anesthesia while mitigating some of the deleterious effects of this anesthetic on memory and neuronal viability. These findings suggest that flumazenil may be a useful clinical adjunct to anesthetic practice to rapidly reverse general anesthesia and to possibly prevent cognitive problems after anesthesia.



Disclosures: J.A. Fidler: None. **B.L. Raymond:** None. **S.C. Burke:** None. **S.R. Baber:** None. **C. Karlapalem:** None. **P.S. Garcia:** None.

581**Poster**

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.01/LLL40

Topic: F.03. Motivation and Emotion

Support: This research is supported by the intramural research program of the National Institute on Aging, NIH.

Title: Functional coupling between non-cholinergic basal forebrain neurons and midbrain dopaminergic neurons

Authors: *I. AVILA, S.-C. LIN;
Neural Circuits and Cognition Unit, LBN, Natl. Inst. On Aging, NIH, Baltimore, MD

Abstract: Recent studies show that the brain uses two major neuromodulatory systems, the midbrain dopaminergic (DA) neurons and non-cholinergic basal forebrain (BF) neurons, to separately encode the hedonic valence and motivational salience of the stimulus. However, motivational salience and hedonic valence are strongly coupled in reward-related contexts because reward-predicting stimuli are highly motivationally salient. This underscores the importance of understanding whether DA neurons and non-cholinergic BF neurons are functionally distinct or functionally coupled in reward-related contexts. Here we investigate this issue by simultaneously recording neuronal activity in the two regions while rats perform a reward-biased simple RT task. In this task, rats were trained to respond to two distinct auditory stimuli that predicted different amounts of reward in the same port. We found that the activity profile of non-cholinergic BF neurons and putative midbrain DA neurons were highly similar throughout different epochs of this task. Both types of neurons showed similar tonic activity modulation in anticipation to stimulus onset, and robust bursting responses at similar latencies to the trial start signal, to both reward-predicting sounds as well as to reward delivery. Furthermore, both types of neurons showed robust bursting responses at similar latencies to the trial start signal, to both reward-predicting sounds as well as to reward delivery. Furthermore, both midbrain DA neurons and non-cholinergic BF neurons showed similar bursting amplitude modulation between the two stimuli that predicted large or small reward, and similar reward prediction error modulation toward reward delivery. These observations suggest that non-cholinergic BF neurons and midbrain DA neurons are not functionally distinct but highly

coupled in the reward-related contexts that we tested. The lack of functional specification between these two neuronal populations suggests a highly coordinated state early in the decision making process involving multiple subcortical neuromodulatory systems. These findings also caution against simple causal inferences based on observing BF or midbrain DA neuronal activity alone.

Disclosures: I. Avila: None. **S. Lin:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.02/LLL41

Topic: F.03. Motivation and Emotion

Support: This research is supported by the intramural research program of the National Institute on Aging, NIH.

Title: Motivational salience signal in the basal forebrain tracks behavioral performance during learning

Authors: *H. MANZUR[1], S.-C. LIN[2];
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Abstract: Recent studies show that a subset of basal forebrain (BF) neurons encodes the motivational salience of stimuli with robust bursting responses. This BF motivational salience signal does not depend on the physical salience of the stimulus and is acquired through learning because the same stimulus does not elicit bursting responses before animals learn about the stimulus-outcome association. However, how the BF motivational salience signal emerges through the learning process remains unknown. In this study, we investigated whether the emergence of the BF bursting response to a novel stimulus occurred before rats learned about the stimulus-outcome association and thus may facilitate learning, or whether the BF bursting response closely tracked behavioral performance during the learning process and therefore reflects the outcome of learning. After a novel stimulus was introduced, we found that the quick expression of the correct behavioral choice toward the novel stimulus was not driven by stimulus-outcome learning, but by a significant shift in behavioral strategy favoring exploration. This shift in behavioral strategy enabled rats to discover and learn about the new stimulus-outcome association early in the learning process and was slowly reversed after several training sessions as the new association was acquired. We found that the BF bursting response to the novel stimulus slowly emerged over several training sessions and closely tracked the reaction

time toward the novel stimulus throughout the learning process. Furthermore, we found that the gradual increase of BF bursting response to the novel stimulus over session was accompanied by a gradual decrease of BF bursting response toward the receipt of reward, resembling a reward prediction error signal. These results support that the BF motivational salience signal closely tracks behavioral performance during the learning process, and suggest that the reward prediction error modulation may serve as a learning signal in reinforcement learning. These observations also highlight the importance of shifting behavioral strategies as a key mechanism during the initial phase of learning.

Disclosures: H. Manzur: None. **S. Lin:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.03/LLL42

Topic: F.03. Motivation and Emotion

Support: NIN/NINDS grant NS023945

Title: The topographic organization of auditory cortical projecting basal forebrain cells

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Abstract: Learning induced plasticity in the primary auditory cortex has been a well-documented phenomenon across species and learning conditions. Learned tones gain representational area in primary auditory cortex (Weinberger, 2007). This increased representation is correlated with learned importance and memory strength and can be thought of as a cortical memory trace. The release of acetylcholine (ACh) is critical for the development of learning induced plasticity in auditory cortex. The basal forebrain cholinergic system consists of large cortically projecting cells, which provide the majority of acetylcholine to the entire cortical mantle where it plays a role in cortical activation, attention as well as plasticity. The heterogeneity and anatomical complexity of the BF has contributed to the idea that the BF is a diffuse cortically projecting system without specificity. However, research has shown that ACh may be released in a modality or area specific fashion in the cortex (Parikh et al., 2007), suggesting an architecture that is not readily expressed in the BF anatomy. Recently, we demonstrated that the BF organization reflects corticocortical connectivity patterns (Zaborszky et al., submitted). In particular, these data show that cortical areas that are interconnected share inputs from an overlapping population of BF neurons. The current experiment tests whether such

an anatomical organization exists for BF cells projecting to auditory cortex. Male Sprague-Dawley rats were anesthetized and a craniotomy was made over the auditory cortex. Auditory cortex was defined electrophysiologically or based on the cortical vasculature. Fluorogold (FG) was then deposited in the auditory cortex via ionotopheresis. A second craniotomy was made over one of several anterior cortical areas including, infralimbic, agranular insular, or auditory responsive insular cortex (defined electrophysiologically) and a small amount of Fast Blue (FB) was pressure injected into the region. The rats survived for one week, were transcardially perfused, brains removed and placed in a 30% sucrose solution. The brains were then sectioned (50 μm), immunostained for choline acetyl transferase and assessed for the presence of FG and FB retrograde labeling in the BF. Preliminary data suggests that the auditory cortex shares an overlapping pool of projection cells within the BF, specifically, with the auditory responsive granular insular cortex. These data support the hypothesis that the BF is not a diffuse system and suggests that such an organization can co-activate distinct cortical areas that are functionally linked.

Disclosures: C.M. Chavez: None. **L. Zaborszky:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.04/LLL43

Topic: F.03. Motivation and Emotion

Support: NIMH R01 MH039683

NHLBI HL095491

NIMH R21 MH094803

Title: Cholinergic neurons excite cortically-projecting basal forebrain GABAergic/parvalbumin neurons

Authors: *C. YANG, J. T. MCKENNA, R. E. BROWN;
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Abstract: Drugs enhancing cholinergic activity are used to treat the defective cortical activation observed in disorders such as Alzheimer's disease and schizophrenia. These drugs act directly on neocortical neurons to promote fast, rhythmic, electrographic activity, mimicking cholinergic input from the basal forebrain (BF). However, whether cholinergic neurons also affect other BF

cortically projecting neurotransmitter systems is unknown. One group of BF neurons of particular interest are GABAergic neurons containing parvalbumin (PV), since they are fast-firing and have projections to cortical PV interneurons involved in generating gamma band oscillations (GBO). Furthermore, we have recently found that optogenetic stimulation of these neurons entrains cortical GBO. Here we demonstrate using immunohistochemistry in transgenic mice expresing green fluorescent protein in GABAergic neurons (GAD67-GFP knock-in mice) that BF cholinergic neurons are intermingled with GABAergic neurons and vesicular acetylcholine transporter-positive cholinergic fibers appose GABAergic neurons containing PV. Pharmacological activation of cholinergic receptors with carbachol (50 μM) increased the firing rate of cortically-projecting BF GABAergic neurons, via activation of M1/3 muscarinic receptors and sodium-permeable cation channels. Furthermore, optogenetic stimulation of cholinergic neurons caused a muscarinic receptor-mediated (atropine-sensitive) inward current in putative GABAergic neurons. Thus, the cortical activating and pro-cognitive actions of cholinergic agents may be mediated in part by activation of cortically projecting BF GABAergic neurons, a potential novel target to treat disorders of cortical activation.

Disclosures: C. Yang: None. **J.T. McKenna:** None. **R.E. Brown:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.05/LLL44

Topic: F.03. Motivation and Emotion

Support: NIH/NINDS Grant NS023945

Title: Visualization of basal forebrain cholinergic clusters in Cartesian coordinate system and investigation of their functional significance

Authors: *P. GOMBKÖTO, L. ZABORSZKY;
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Abstract: Cholinergic corticopetal neurons in the basal forebrain (BF) show inhomogeneous distribution where dense clusters of neurons are interrupted by regions of low cellular density in human as well as in rodents and monkeys (Zaborszky et al., 2008). Using a buble-clustering algorithm we have recently described that the distribution of clusters is not random, but show similar pattern across rat subjects (Nadasdy et al., 2010). A preliminary analysis of the cortical targets of the clusters suggest that cell clusters in the BF project only to a small subset of cortical areas that most likely are interconnected. The prerequisite to investigate BF cluster function, we

need to determine the location of clusters in a Cartesian coordinate system with reference to the Paxinos rat atlas (i) and design a digital large-scale recording system (ii) that would allow selective stimulation of BF clusters and record multiple cortical targets. Here we report our preliminary results in both technical directions.
We developed a Matlab program as a clustering function which can determine the existing clusters within Euclidean space where the cells positions are in Cartesian coordinates. We confirmed the inhomogeneity of the cholinergic system, defined the cell clusters, quantified and localized them with reference to the Paxinos coordinates.
For the second aim we are developing a large-scale recording system that can monitor spikes or local field potentials (LFP) with extreme high spatial and temporal resolution with the possible lowest restriction of the free movement of the animals. The digital multi-channel amplifier is using a Digital Electrophysiology Interface Chip provided by Intan Technologies. Digitalization takes place in the head stage that is directly connected to output of silicon electrodes on the head of the animal. The 32 channels are compressed/digitalized in only two cables (LVDS signals) leaving the head stage. Our new main board with a field-programmable gate array (FPGA) is able to control 32 Intan chips which allows simultaneous processing of 1024 channels by 30Khz/channel sampling rate. The main board transmits digital information to the PC through a regular Ethernet, therefore, there is no need for various digitalization cards since we utilize the PC's already existing resources. FPGA makes several solutions available, including the selection of specific channels for biofeedback, and real time spike sorting.
These new infrastructural developments could lay down the foundations for exploring new avenues in understanding the functional architecture of basalo-cortical networks.

Disclosures: P. Gombköto: None. **L. Zaborszky:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.06/LLL45

Topic: F.03. Motivation and Emotion

Support: Foundation for Science and Technology, Portugal

NIH R01 NIDCD DC12557

Title: Neuromodulatory plasticity governs cortical plasticity

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Neurosciences and Cell Biology, Univ. of Coimbra, Coimbra, Portugal; [3]Ctr. for Neural Sciences, NYU, New York, NY

Abstract: The cerebral cortex is plastic, dynamically representing the external environment. Cortical networks are part of neural circuits that include subcortical neuromodulatory systems, that contextualize incoming sensory stimuli. Neuromodulator release is required for cortical plasticity, but it is also possible that modulator systems themselves are plastic, providing differential modulatory control based on past experience.

Here we investigate how the noradrenergic locus coeruleus interacts with cortical circuits and enables plasticity. Adult rats were anesthetized, stimulation electrodes implanted in the locus coeruleus, and primary auditory cortex identified. In vivo whole-cell recordings (voltage-clamp or current-clamp) were made from auditory cortical neurons or locus coeruleus neurons, and pure tones of varying frequencies and intensities were presented to the animal to characterize auditory responses.

Pairing tones with locus coeruleus activation greatly increased synaptic and spiking responses by two- to ten-fold, elevating and flattening tuning curves across frequencies. Eventually, over tens of minutes, tuning curve structure returned, leaving the paired frequency selectively enhanced. Multiple recordings after a single episode of pairing demonstrated that tuning changes stabilized after 3-6 hours and persisted for 11+ hours.

Blocking noradrenalin receptors only during pairing temporarily blocked cortical effects, but blocking receptors after pairing prevented expression of long-term changes. This suggested that release of noradrenalin from locus coeruleus neurons was changed post-pairing, and might persist for hours. In vivo whole-cell recordings from locus coeruleus neurons showed that pairing induced long-term changes in noradrenergic circuitry, as they began to respond to paired stimuli with short latency (~30-50 msec). These changes were prevented by infusion of the NMDA receptor antagonist APV in locus coeruleus, which also reduced the duration of cortical plasticity (from 11+ hours to ~3 hours).

Furthermore, rats were implanted with locus coeruleus stimulation electrodes and trained to respond to a target 4 kHz tone, for a food reward. Locus coeruleus pairing increased detection of quiet tones at the expense of recognition of target from unrewarded foil tones. Even after a single pairing episode, improvements in detection could last for days or months in some animals.

Our results demonstrate that plasticity of subcortical neuromodulation has a major impact on the dynamics of cortical plasticity, creating persistent changes in sensory perception.

Disclosures: A. Martins: None. **R.C. Froemke:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.07/LLL46

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01NS073125

Title: Population coding of stimulus and reward in rat basal forebrain

Authors: *E. E. THOMSON[1], K. SYLVESTER[2], Â. TAKIGAMI[2], J. LOU[3], M. NICOLELIS[2];
[1]Duke Univ., DURHAM, NC; [2]Neurobio., Duke, Durham, NC; [3]Keck Sch. of Med., USC, Pasadena, CA

Abstract: The basal forebrain (BF) is known to encode stimulus salience, and it also plays important roles in neural plasticity and attention. To determine how BF activity relates to stimulus and reward parameters, we recorded from populations of single units in the nucleus basalis of Meynert of rats as they performed a whisker-dependent aperture-width discrimination task. As expected, individual neurons and populations encoded reward values with high accuracy during the task. Additionally, within 100 ms of stimulus onset, BF populations carried significant levels of information about the tactile stimulus. This is in contrast to what we observed in lightly anesthetized animals: when we delivered the same stimuli to the whiskers of lightly anesthetized animals, BF neurons transmitted significantly less information about the stimuli. Such results suggests that in the awake behaving animal, information about the stimulus is routed to BF and could be part of a mechanism for selective attention.

Disclosures: E.E. Thomson: None. **M. Nicolelis:** None. **K. Sylvester:** None. **Â. Takigami:** None. **J. Lou:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.08/LLL47

Topic: F.03. Motivation and Emotion

Support: NSF Grant 0910485

Title: Basal forebrain neuronal activity maps the sequences of locomotor actions associated with fluid traversal of complex paths through an environment

Authors: *S. KOLBU, A. A. CHIBA, D. A. NITZ;
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Abstract: Fast, fluid locomotion along complex paths through an environment demands an ongoing integration of sensory information with current and planned locomotor states. It has been suggested that the route-centered spatial firing patterns of posterior parietal cortex neurons in behaving animals could provide a temporal framework for such integration, through projections to primary and secondary sensory cortices as well as to the medial precentral sub-region of prefrontal cortex, an area having neuronal activity related to both present and future actions. The substantia innominata and ventral pallidal sub-regions of the basal forebrain, through the cortical projections of their GABAergic and cholinergic neuron populations, are also in a position to coordinate the ongoing sensorimotor integration associated with path running. Basal forebrain neurons may contribute in a variety of ways to the execution of a series of actions through environmental space. As seen in prior work, they could respond primarily to specific sensory stimuli, or to rewards associated with task performance. They could instead simply provide a tonic excitation of specific cortical regions. Alternatively, they could produce unique patterns of activity for each path position, or they could generate activity patterns associated with specific locomotor actions. In the present study, the form by which basal forebrain neurons may participate in navigation along a path was determined by examining the firing dynamics of rat basal forebrain neurons during traversal of tracks with varying degrees of path complexity and recurrence of path sub-components. Among the recorded population of basal forebrain neurons in seven animals, a sub-population was found which exhibited activity peaks closely related to specific behaviors associated with path running (e.g., left versus right turns). This result is consistent with a role for the basal forebrain in coordinating streams of sensory information with present and planned actions in the context of complex action sequences.

Disclosures: S. Kolbu: None. **A.A. Chiba:** None. **D.A. Nitz:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.09/LLL48

Topic: F.03. Motivation and Emotion

Support: NSF 0910485

Title: Cell assemblies of the basal forebrain exhibit beta-frequency dynamics

Authors: *D. TINGLEY, A. ALEXANDER, S. KOLBU, A. CHIBA, D. NITZ;
UCSD, La Jolla, CA

Abstract: The basal forebrain (BF) consists of a heterogeneous population of cholinergic, GABAergic, and glutamatergic neurons having projections to many cortical regions. The importance of these projections for cognitive function has been demonstrated in behavioral studies employing lesions of BF and in examining impairments in cognitive processing in patient populations (e.g., Alzheimers disease). In recent years, such work has been complemented by single neuron recording studies demonstrating that BF cell populations: 1) form activity patterns for all phases of a selective attention task; 2) consist of neuron populations having distinct inter-spike interval (ISI) patterns; 3) are responsive to salient sensory stimuli and rewards; and 4) exhibit complex task-phase-specific firing patterns paralleling those observed in their efferent targets (e.g., parietal cortex). These functional and anatomical features of the BF allow for the possibility of complex neural assemblies that integrate information, and modulate cortex, over a distinct time frame.

BF neurons were recorded while rats performed a visual-spatial attention task. Specific groups of neurons within the recorded population had ISI histograms that represent either 'bursty' or tonic firing. These groups are most active at different phases of a behavioral task, representing temporally, and perhaps functionally, distinct cell assemblies. Such shifts in activity patterns may represent a switching mechanism between network attractor states coinciding with attentional shifts across phases of a complex behavioral task.

To further characterize interactions among BF neurons during task performance, spike firing patterns among pairs were examined in the context of the 'cell assembly' hypothesis. Using a generalized linear model, spike times of BF neurons were more accurately predicted by temporally smoothed spike time probabilities of simultaneously recorded peer neurons. Additionally, BF neurons exhibited an optimal temporal window for synchronous spike generation of 30-80 ms (12-33 Hz). This indicates that BF cell assemblies are synchronized over a beta-frequency time scale which has been proposed as an optimal global synchronization frequency.

In addition to the anatomical connections between the BF and cortex, the electrophysiological properties of the BF further suggest temporally precise integration, synchronization, and modulation of cortical targets during a task that demands visual-spatial attention. These findings suggest that the temporal dynamics of cell assemblies in the BF differ from previously explored brain regions, as does its functional and behavioral importance.

Disclosures: D. Tingley: None. **A. Alexander:** None. **S. Kolbu:** None. **A. Chiba:** None. **D. Nitz:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.10/LLL49

Topic: F.03. Motivation and Emotion

Support: NIH Grant NS023945

NSF Grant SBE 0542013

Title: Visuo-motor versus somato-motor integration in the rat basal forebrain

Authors: *M. R. GIELOW[1], L. K. QUINN[3], J. M. CONNER[4], A. A. CHIBA[3], K. D. ALLOWAY[5], L. ZABORSZKY[2];
[2]Neurosci., [1]Rutgers Univ., Newark, NJ; [3]Cognitive Sci., [4]Neurosci., UCSD, La Jolla, CA; [5]Ctr. for Neural Engin., Penn State Univ., University Park, PA

Abstract: Sensory-motor integration is key to many categories of learning, yet the mechanisms of this integration are disputed. The cholinergic basal forebrain (BF; 'nucleus basalis') is a facilitator of cortical plasticity, and its efferents differentially modulate motor, somatosensory, and visual cortical activity via topographically organized projections. During various learning tasks, sensorimotor integration occurs concurrently with BF-mediated cortical plasticity. In order to query the extent of the role of the BF in sensorimotor integration, we first identify candidate sites within the BF that might jointly modulate primary sensory and motor areas. This is achieved by depositing distinct retrograde tracers in physiologically-identified primary visual, motor, and somatosensory areas, and subsequently staining forebrain sections for choline acetyltransferase (ChAT). Although a given group of cells projecting to a particular cortical area tends to occupy several of the classical cytoarchitectural subdivisions of the BF, for a simple quantitative analysis we express the overlap and segregation of sensory-versus-motor projecting somata within each of the major BF regions, including the medial septum, diagonal band, substantia innominata, ventral pallidum, and globus pallidus / internal capsule. The resulting maps reveal locations to be targeted in future subregional and cell-type-specific manipulations of the ascending basal forebrain system during behavioral tasks, and indicate that for purposes of probing sensory-motor integration at the level of the BF, simple stimulation of the nucleus basalis is likely to be insufficient.

Disclosures: M.R. Gielow: None. **L.K. Quinn:** None. **J.M. Conner:** None. **A.A. Chiba:** None. **K.D. Alloway:** None. **L. Zaborszky:** None.

Poster

581. **Basal Forebrain: Neurophysiology and Function**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 581.11/LLL50

Topic: F.03. Motivation and Emotion

Support: NIH/NIA Intramural Research Program

NIH Grant PO1 AG09973-19

Title: Better late than never - the role of basal forebrain inhibition in successful and failed stopping in the stop signal task

Authors: *J. D. MAYSE[1], G. NELSON[2], I. AVILA[2], M. GALLAGHER[1], S.-C. LIN[2]; [1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Lab. of Behavioral Neurosci., Natl. Inst. on Aging, Baltimore, MD

Abstract: Cognitive inhibitory control, or the ability to suppress responses inappropriate for the context, is essential for flexible and adaptive behavior. One powerful paradigm for studying inhibitory control is the Stop-Signal Task (SST), which requires subjects to suppress a response to a go-signal when it is occasionally followed by a stop-signal. The SST is unique in that it allows for quantitative estimation of the time required to suppress the planned go-response, known as stop-signal reaction time (SSRT). We recently identified a novel subcortical neural correlate of the SSRT in the basal forebrain (BF). In rats performing a SST, we found that BF neurons with bursting responses to the go-signal (tone) were selectively inhibited by the stop-signal (light). The onset of BF neuronal inhibition was correlated with and preceded SSRT by ~30ms. In support of a causal relationship, artificially inducing BF neuronal inhibition reproduced stopping behavior even in the absence of the visual stop-signal.
In the current study, we examined whether and how BF activity was modulated between successful and failed stop trials, a comparison commonly used to identify neural mechanisms that lead to successful inhibitory control. We found that the stop signal was similarly processed on failed and successful stop trials prior to the estimated SSRT. As a result, failure to stop was not associated with absent or diminished BF inhibition, but instead with BF inhibition arriving too late after the initiation of the go response. Nevertheless, the presence of BF inhibition in failed stop trials, both in SST and in artificially induced BF inhibition, had a strong influence on behavior and resulted in corrective reentry responses especially when go responses were initiated just before SSRT. Together, our results show that successful or failed stopping is not driven by differential engagement of inhibitory control mechanism but largely determined by the variability in the go-process. The inhibitory control mechanism in the BF represents a robust and invariant process that is always engaged irrespective of behavioral outcome, which strongly modulates behavior even when it was too late to stop.

Disclosures: J.D. Mayse: None. **M. Gallagher:** None. **S. Lin:** None. **G. Nelson:** None. **I. Avila:** None.

582**Poster**

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.01/LLL51

Topic: F.03. Motivation and Emotion

Support: MH050479

MH093412

Title: Ketamine prevents neurochemical and behavioral consequence of uncontrollable stress

Authors: *K. H. KUBALA, J. P. CHRISTIANSON, J. AMAT, D. C. COOPER, L. R. WATKINS, S. F. MAIER;
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Abstract:
Exposure to an acute uncontrollable stressor, such as inescapable tail shock (IS), produces a variety of anxiety-like behaviors that depend on intense activation of serotonergic neurons in the dorsal raphe nucleus(DRN), which leads to exaggerated release of serotonin (5-HT) in anxiety related projection regions, such as the basolateral amygdala (BLA), this being critical to the mediation of the behavioral changes. Recent evidence suggests that ketamine, an NMDA receptor antagonist, produces rapid antidepressant-like effects in patients diagnosed with depression, and animal studies have shown that it may block some of the behavioral and physiological changes produced by stressors. The goal of the present study was to determine whether ketamine would mitigate the behavioral consequences of IS and the intense activation of the DRN produced by IS. Ketamine injected i.p. either two hours before, or immediately after IS, blocked the typical stress-induced decrease in social exploration. This behavioral deficit is dependent on serotonergic activation of the BLA, via afferents from the DRN. Ketamine also completely blocked the large serotonergic influx in the BLA produced by IS when administered 2 hours before the stressor. These results provide evidence that ketamine may block behavioral effects of IS by acting on the 5-HT system in the DRN.

Disclosures: K.H. Kubala: None. **J.P. Christianson:** None. **J. Amat:** None. **D.C. Cooper:** None. **L.R. Watkins:** None. **S.F. Maier:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.02/LLL52

Topic: F.03. Motivation and Emotion

Support: MH050479

MH093412

Title: Controllable versus uncontrollable aversive stimuli differentially trigger ERK signaling in the dorsal striatum

Authors: *R. A. DAUT[1], J. P. CHRISTIANSON[1], J. G. N. FLYER[2], L. R. WATKINS[2], S. F. MAIER[2];
[1]Dept. of Psychology and Neurosci., [2]Univ. of Colorado, Boulder, CO

Abstract: The action-outcome learning system shapes goal-directed behavior. The medial prefrontal cortex and dorsal striatum form an action-outcome circuit that has typically been studied using positive rewards such as food. Here we attempt to extend the function of this circuit to a type of instrumental learning that involves the removal of an aversive stimulus, i.e. negative reinforcement. Rats were exposed to a series of tailshocks that could be terminated by making a wheel-turn response (controllable shock). A second group of rats was yoked to the first and received equivalent tailshocks which could not be terminated (uncontrollable shock). Rats were sacrificed immediately after 0, 25 or 100 trials, brains dissected, micro-punches taken from both the medial and lateral dorsal striatum (DMS/DLS) and prepared for Western blot analysis. Expression of phosphorylated extracellular regulated kinase 1 and 2 (pERK1/2) and phosphorylated p70S6K were quantified relative to their total isoforms. Exposure to 25 controllable shocks led to a significant elevation in DMS pERK1 levels relative to unshocked controls. In comparison, exposure to 100 uncontrollable tailshocks increased pERK2 in both DMS and DLS and increased pERK1, but only in the DMS. Phosphorylated p70S6K was increased by 100 shocks but did not appear to be sensitive to control. These results suggest that activation of the ERK mediated pathway may play a role in the behavioral consequences of uncontrollable shock, i.e. learned helplessness. Mechanistic studies aimed to test the role of ERK1/2 in the effects of controllable versus uncontrollable stress are ongoing.

Disclosures: R.A. Daut: None. **J.P. Christianson:** None. **S.F. Maier:** None. **J.G.N. Flyer:** None. **L.R. Watkins:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.03/LLL53

Topic: F.03. Motivation and Emotion

Support: MH050479

MH093412

Title: The long-lasting protective effects of controllable stress require ERK in the medial prefrontal cortex

Authors: J. G. N. FLYER[1], *J. P. CHRISTIANSON[2], L. R. WATKINS[1], S. F. MAIER[1]; [1]Psychology & Neurosci., Univ. of Colorado, Boulder, CO; [2]Psychology and Neurosci., Univ. of Colorado, BOULDER, CO

Abstract: Exposure to escapable tailshocks (ES) each of which can be terminated by a behavioral response is a behaviorally controllable stressor that confers protection from the neurochemical and behavioral consequences of future inescapable tailshock (IS), an uncontrollable stressor. This phenomenon, termed "behavioral immunization", requires NMDA-receptor dependent plasticity within the medial prefrontal cortex (mPFC). In the present studies we sought to test the involvement of the mitogen activated protein kinase 1/extracellular regulated kinase 1 and 2 (MAPK1/ERK1,2) and the mammalian target of rapamycin (mTOR) molecular cascades in the behavioral immunization paradigm. Using a combination of Western blot and immunohistochemistry, we identified a rapid and significant increase in phosphorylated ERK1 and ERK2 within the prelimbic cortex in rats exposed to controllable ES. Exposure to exactly equal but uncontrollable IS did not alter ERK2 phosphorylation from baseline. To determine whether ERK1/2 signaling is critical to the behavioral immunization phenomenon, the MEK1/2 inhibitor U0126 (5μg/side or vehicle) was injected into the prelimibic mPFC prior to exposure to controllable stress. One week later rats received uncontrollable stress and subsequent testing for anxiety-like behavior in a social exploration test. As previously reported, uncontrollable stress exposure significantly reduced social exploration, but prior experience with controllable stress prevented this reduction. In the critical group, U0126 blocked the behavioral immunization effect. Controllable stress exposure also increased phospho-p70S6K in prelimbic mPFC, suggesting a possible involvement of mTOR. Studies designed to test the possible contribution of mTOR in this paradigm are ongoing. However, existing data clearly implicate ERK pathway activity as critical for the stress-inoculating effect of behavioral control.

Disclosures: J.G.N. Flyer: None. **J.P. Christianson:** None. **L.R. Watkins:** None. **S.F. Maier:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.04/LLL54

Topic: F.03. Motivation and Emotion

Support: NIH grant R24 MH075888-01

Title: The effect of hydrocortisone on emotion regulation neurocircuis in patients with PTSD

Authors: *G. OKADA, S. T. MA, S. HO, S. TAYLOR, J. L. ABELSON, I. LIBERZON; Univ. of Michigan, Ann Arbor, MI

Abstract: In the present study, we examined the effects of hydrocortisone administration on emotion regulation in individuals with and without posttraumatic stress disorder (PTSD) at a neuronal level through fMRI. Twelve patients with PTSD and 12 healthy controls were exposed to a series of compound pictures of faces (angry, fear, neutral) and places(indoor, outdoor) and cued to indicate 1) whether the face was male or female, or 2) whether the background was indoor or outdoor, or 3) whether the subject liked or disliked the feces. The experiment was of a counter-balanced, placebo-controlled, within-subject design. Two days of experiments were scheduled with a minimum interval of 3 days, and 100mg hydrocortisone (HCT) or Placebo was administered. As in our prior works, experimental manipulation robustly activated neurocircuits involved in emotional regulation by shifting attention and cognitive appraisal respectively. Furthermore, differential cortisol modulations of task related activation were observed in the hippocampus and subgenual anterior cingulate cortex between in controls and in patients with PTSD. These results suggest the altered glucocorticoid sensitivity in emotion regulation neurocircuit in PTSD.

Disclosures: G. Okada: None. **S.T. Ma:** None. **S. Ho:** None. **S. Taylor:** None. **J.L. Abelson:** None. **I. Liberzon:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.05/LLL55

Topic: F.03. Motivation and Emotion

Support: NIH grant R24 MH075888-01

Title: Cortisol modulation on emotion regulation neurocircuits

Authors: *S. T. MA[1], G. OKADA[3], S. HO[2], S. TAYLOR[2], J. L. ABELSON[2], I. LIBERZON[2]; [1]Dept of Psychiatry, [2]Univ. of Michigan, Ann Arbor, MI; [3]Hiroshima Univ., Hiroshima, Japan

Abstract: This study examined the effects of cortisol administration on emotion regulation at a neuronal level through fMRI. Forty individuals participated in this study and were randomly assigned to hydrocortisone (10 males, 10 females) and placebo control groups (10 males, 10 females). We used the shifted-Attention Emotion Appraisal Paradigm to investigate the neurocircuits related to emotion processing and regulation. In this task, subjects were exposed to a series of compound pictures of faces (angry, fear, neutral) and places(indoor, outdoor) and cued to indicate the (i) gender of the face (implicit emotional processing), (ii) indoor/outdoor of the place (shifted attention to places), or (iii) whether the face is liked or disliked (cognitive appraisal). As in our prior works, the experimental manipulation robustly activated neurocircuits involved in emotional processing and regulation. In addition, hydrocortisone administration significantly enhanced the medial prefrontal cortex activation ($p = 0.038$) which was increasingly activated cognitive appraisal > emotion processing > shifting attention to places. These results suggest the direct nongenomic effects of cortisol on emotion regulation neurocircuit.

Disclosures: S.T. Ma: None. **G. Okada:** None. **S. Ho:** None. **S. Taylor:** None. **J.L. Abelson:** None. **I. Liberzon:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.06/LLL56

Topic: F.03. Motivation and Emotion

Support: KAKENHI 23300130

KAKENHI 22790239

Title: Opposing roles of corticotropin-releasing factor and neuropeptide Y within the dorsolateral bed nucleus of the stria terminalis in the negative affective component of pain in rats

Authors: *S. DEYAMA[1], S. IDE[1], A. OHNO[1], R. TAMANO[1], K. KOSEKI[1], T. NAKA[1], C. MARUYAMA[1], M. YOSHIOKA[2], M. MINAMI[1];
[1]Dept Pharmacol, Grad Sch. Pharm Sci., Hokkaido Univ., Sapporo, Japan; [2]Dept Neuropharmacol, Grad Sch. Med., Hokkaido Univ., Sapporo, Japan

Abstract: Pain is a complex experience consisting of sensory and affective components. Although the neural systems of the sensory component of pain have been studied extensively, those of its affective component remain to be determined. In the present study, we examined the effects of corticotropin-releasing factor (CRF) and neuropeptide Y (NPY) within the dorsolateral bed nucleus of the stria terminalis (dlBNST) on pain-induced aversion and nociceptive behaviors in male Sprague-Dawley rats to elucidate the roles of these peptides in affective and sensory components of pain. In vivo microdialysis showed that intraplantar formalin-evoked pain enhanced the release of CRF in this brain region. Using a conditioned place aversion (CPA) test, we found that intra-dlBNST injection of NBI27914, a CRF1 receptor antagonist, or antisauvagine-30, a CRF2 receptor antagonist, dose-dependently suppressed formalin-induced CPA (F-CPA). These antagonists did not affect formalin-induced nociceptive behaviors. Intra-dlBNST injection of CRF dose-dependently produced CPA even in the absence of noxious stimulation. On the other hand, intra-dlBNST injection of NPY dose-dependently suppressed F-CPA without affecting nociceptive behaviors. Intra-dlBNST injection of NPY also attenuated intra-dlBNST CRF-induced CPA. This inhibitory effect of NPY was blocked by co-administration of BIBP3226, a NPY Y1 receptor antagonist, or L-152,804, a NPY Y5 receptor antagonist. Taken together, these results suggest that CRFergic neurotransmission within the dlBNST plays an important role in pain-induced aversion and that NPY may alleviate pain-induced aversion via the inhibition of CRF-mediated neurotransmission in this brain region.

Disclosures: S. Deyama: None. **S. Ide:** None. **A. Ohno:** None. **R. Tamano:** None. **K. Koseki:** None. **T. Naka:** None. **C. Maruyama:** None. **M. Yoshioka:** None. **M. Minami:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.07/LLL57

Topic: F.03. Motivation and Emotion

Support: KAKENHI 23300130

KAKENHI 22790239

Title: Opposing effects of corticotropin-releasing factor and neuropeptide Y on neuronal excitability in the dorsolateral bed nucleus of the stria terminalis

Authors: *M. MINAMI, T. HARA, S. IDE, K. KANEDA;
Dept Pharmacol, Grad Sch. Pharm Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Using a conditioned place aversion (CPA) test, we found the opposing roles of corticotropin-releasing factor and neuropeptide Y within the dorsolateral bed nucleus of the stria terminalis (dlBNST) in pain-induced aversion. In the present study, to investigate the cellular mechanisms of CRF-induced CPA and counteracting effect of NPY, we examined the effects of these peptides on neuronal excitability in the dlBNST slices prepared from Sprague-Dawley rats using whole-cell patch-clamp recordings. Interestingly, we found that only type II, not type I or III, neurons responded to both CRF and NPY in the dlBNST. CRF depolarized membrane potentials in type II neurons. In contrast, NPY induced hyperpolarization in type II neurons. Furthermore, co-application of NPY extinguished the CRF-induced depolarization. Similar results were observed in firing activities of type II dlBNST neurons. Specifically, CRF increased firing activities of type II neurons, and co-application of NPY suppressed them. These counteracting effects of CRF and NPY on neuronal excitability in type II dlBNST neurons may explain the opposing roles of these peptides in pain-induced aversion. Analyses of I-V relationships demonstrated that there might be at least two classes of type II dlBNST neurons, which we designated as type IIa and type IIb. Decreased potassium conductance and increased non-selective cation current could be involved in the polarizing effect of CRF on type IIa and type IIb neurons, respectively. I-V relationship analyses revealed that NPY reduced steady-state currents, the reversal potential of which was around -60 mV, indicating suppression of cationic conductance(s). ZD7288, an Ih blocker, occluded the hyperpolarizing effect of NPY, demonstrating that NPY-induced hyperpolarization was mediated by blocking Ih channels.

Disclosures: M. Minami: None. **T. Hara:** None. **S. Ide:** None. **K. Kaneda:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.08/LLL58

Topic: F.03. Motivation and Emotion

Support: R01 MH045573

Title: Postnatal refinement of OFC (area 11) projections to the striatum

Authors: *B. K. FAHRENTHOLD[1], S. N. HABER[2];
[1]Neurosci., [2]Pharmacol. and Physiol., Univ. of Rochester, Rochester, NY

Abstract: The orbital frontal cortex (OFC)-striatal circuit plays an integral role in reward-based incentive learning. Dysfunction of this circuit has been implicated in several diseases arising during adolescence, including obsessive-compulsive disorder, addiction, and attention-deficit hyperactivity disorder. In normal development there is a time-window of plasticity where cortico-striatal connection refinement continues postnatally for several months (DiFiglia et al., 1980). This period of plasticity is especially vulnerable. In adult rhesus macaques, dense and patchy terminal fields from area 11, a rostral medial part of OFC, are located ventrally and continue up the medial wall in the rostral caudate. Diffuse terminals surround and extend away from these dense terminal fields. We hypothesize that there are changes in density and distribution of terminal fields from area 11, to the striatum coupled with different developmental ages.
Anterograde tracers were placed into area 11 of rhesus macaques at ages of 3 weeks, 3 months, 6 months, and adult. Dense projection fields and diffuse terminals in the striatum (Haber et al., 2006) were charted using Neurolucida (MicroBrightfield, Colchester, VT). Preliminary observations show two types of terminal fields: dense fields that occur in patches surrounded by more diffuse terminals. Terminal field locations are similar to those seen in adult macaques. However, dense terminal fields at 3 weeks of age occupy a smaller striatal area and diffuse terminals surrounding these dense fields were less extensive compared to adults. This suggests terminations from area 11 to the striatum at early postnatal periods undergo expansion or refinement that extends both the dense and diffuse terminal field occupancy until the adult-like pattern of corticostriatal terminals is reached.
Dense projection fields from OFC converge with those from ventromedial prefrontal cortex, dorsal anterior cingulate cortex, and dorsolateral prefrontal cortex in the adult rostral striatum (Haber et al., 2006). In very young animals, smaller dense projection fields from area 11 appear to be less extensive compared to adults. This suggests convergence of these different cortical areas at early postnatal periods may be less extensive than in the adult, possibly resulting in less integration occurring between functional circuits. However, additional experiments are necessary to address the development of other prefrontal-striatal projections.

Disclosures: B.K. Fahrenthold: None. **S.N. Haber:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.09/LLL59

Topic: F.03. Motivation and Emotion

Support: P50 MH086400

Title: The cingulum bundle contains five distinct segments: Implications for default mode network activity and psychiatric disorders

Authors: *S. R. HEILBRONNER, S. N. HABER;
Pharmacol. and Physiol., Univ. of Rochester, Rochester, NY

Abstract: For centuries, the cingulum bundle (CB) has been recognized as one of the major fiber pathways of the brain. However, its composition and function remain unclear. Diffusion imaging shows that it is abnormal in a variety of psychiatric disorders, including major depressive disorder (MDD) and obsessive-compulsive disorder (OCD). Intriguingly, the CB is also an effective target for ablative neurosurgical treatment (cingulotomy) for these two disorders. Thus, understanding the nature of the fiber projections through the CB will generate insights into OCD, MDD, and their treatments. We injected tracers into more than two dozen subcortical and cortical (both cingulate and non-cingulate) targets. We mapped the resulting efferent fiber pathways through the CB and their terminals. In doing so, we were able to segment the cingulum bundle into five distinct regions: subgenual, rostral dorsal, middle dorsal, caudal dorsal, and temporal.
We observed that all prefrontal fibers projecting to the posteromedial cortex (precuneus, retrosplenial, and posterior cingulate cortex), even those terminating in the relatively dorsal precuneal cortex, passed through the middle and caudal dorsal CB, but did not continue into the temporal CB. The posteromedial cortex is one of the two hubs of the default mode network (the other being the medial prefrontal cortex), a set of regions consistently deactivated by effective task performance. Our observations indicate that all of the direct connections between these two hubs project through the middle and caudal dorsal CB. Based on this, we mapped the projections from different regions within the prefrontal cortex to the posteromedial cortex. Finally, we discuss these results as they relate to neuroimaging data from OCD and MDD patients. These results have the potential to shed light on the relationship between default mode network activity, the cingulum bundle, and psychiatric disorders.

Disclosures: S.R. Heilbronner: None. **S.N. Haber:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); Pfizer, Inc, Eli Lilly and Company, Medtronic, Inc.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.10/LLL60

Topic: F.03. Motivation and Emotion

Support: DGAPA-PAPIIT IN302512-3

Title: Conditioning taste aversion after a chronic stress exposure in rats

Authors: *A. RUIZ GARCIA[1], P. TORRES-CARRILLO[2], I. ROSEMBERG-GARCIA[2], D. PAZ-TREJO[2], H. SANCHEZ-CASTILLO[2];
[1]Psychophysiology, UNAM, Mexico DF, Mexico; [2]Psychophysiology, UNAM, Mexico City, Mexico

Abstract: It has been show that chronic stress exposure causes cognitive deficits, for example, it has been observed a poor performance in memory tasks and the impairment on acquisition and extinction this probably because a decrease in the glucocorticoid receptors of the hippocampus and affectation of the cholinergic system. Those results have been related to an impairment of the prefrontal cortex. Also it has been demonstrated that the exposure to stressor before the presentation of an aversive stimuli facilitates the learning. The aim of this study was to evaluate the effect of chronic exposure to different stressors in Conditioning Taste Aversion (CTA) using a cholinergic agonist (nicotine) to observe how changes would be given in associative learning. We used 7 male Wistar rats with 4 months old at the beginning of the experiment. The subjects were exposed to 7 days of chronic stress with a variable stressors battery, this consisted of placing the rats for 12 hours (overnight) in humid sawdust for two days, five minutes of swimming in cold water (16 ° C) for three days and finally, were exposed to 12 hours (overnight) to lights on for two days, all of those were randomizing along the experiment. The animals were tested to Conditioning Taste Aversive (CTA), this consisted in exposed the rats to saccharin 30 minutes per day, after that were injected with nicotine at a dose of 1.6 mg / kg to cause the aversion for 4 days. The CTA extinguish time was recorded. The obtained results showed that the animals began to consume saccharin after 8 days, however not returned to the basal levels, and the time to acquired extinction took 8 sessions. This suggests that expose to chronic different stressors in rats demonstrated aversive learning is better consolidated, it has also been observed in people who had experience a traumatic event due to they remembered the context related to the aversive situation and they generalize it to every environment.

Disclosures: A. Ruiz Garcia: None. **P. Torres-Carrillo:** None. **I. Rosemberg-Garcia:** None. **D. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.11/LLL61

Topic: F.03. Motivation and Emotion

Support: NINDS grant T32 NS07224

Title: Investigation of the neural correlates of emotion regulation with and without explicit instruction to 'reappraise'

Authors: *D. Z. BOLLING, E. KRAPOHL, N. PITSKEL, K. PELPHREY;
Yale Child Study Ctr., New Haven, CT

Abstract: Functional MRI research on emotion regulation has become increasingly common in healthy and clinical populations. Most of this work has focused on cognitive reappraisal, which involves an effortful reinterpretation of an emotion-inducing stimulus in order to modify one's response. While this work focuses on directed reappraisal, regulation in a naturalistic setting is almost always uninstructed. The current study compares directed reappraisal with an identical task that facilitates spontaneous regulation by either giving the participant information that the forthcoming picture will be negative, or leaving the valence ambiguous by telling the participant that the forthcoming picture may be negative or neutral. We hypothesize that warning subjects of the impending negative image will enable spontaneous regulation, reflected in neural responses resembling those of instructed reappraisal.

To investigate neural correlates of directed cognitive reappraisal in healthy adults, we used fMRI to measure activation to disgust images under three instruction conditions, "look", "decrease", and "increase." Neutral images were included in the "look" condition. To investigate neural correlates of spontaneous regulation in a matched group of healthy adults, we presented identical disgust images under two instruction conditions, "certain" and "ambiguous." Neutral images were included in the "ambiguous" condition. In each trial, an instruction was given, followed by an image presented for 3 seconds, concluding with an affect rating screen.

Brain responses to instructed cognitive reappraisal of negative images (decrease > look) in 20 healthy adults ($p < 0.05$) revealed increased activation during disgust image viewing following the "decrease" instruction (versus "look") in dorsolateral and ventrolateral prefrontal cortices, as well as dorsomedial prefrontal cortex, consistent with past work defining the neural correlates of reappraisal. In addition, reappraisal was associated with decreased activation in right amygdala and bilateral insula. In a second group of 12 healthy adults ($p < 0.05$), brain responses to disgust image viewing following a "certain" instruction (versus "ambiguous") revealed increased activation in dorsolateral prefrontal cortex, as well as decreased activation in insula.

Results suggest overlap in the neural correlates of instructed emotion regulation and anticipatory apprehension of disgust images, which may be indicative of spontaneous regulation in cases

when one knows of an impending negative stimulus. The relationship between spontaneous regulation and emotion regulation styles will be tested in a larger sample.

Disclosures: D.Z. Bolling: None. **E. Krapohl:** None. **N. Pitskel:** None. **K. Pelphrey:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.12/LLL62

Topic: F.03. Motivation and Emotion

Title: Low-frequency rTMS over the ventrolateral prefrontal cortex modulates reappraisal-based down-regulation of negative affect

Authors: *J. U. KIM, S. R. LEVINE, L. R. BLAIR, D. H. ZALD;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Previous neuroimaging studies have demonstrated that successful reappraisal-based regulation of negative affect is associated with greater ventrolateral prefrontal cortex (vlPFC) recruitment. However, it is still unclear whether vlPFC activity plays a causal role in the reappraisal-based emotion regulation process. In the current study, we sought to investigate the effect of low-frequency repetitive transcranial magnetic stimulation (rTMS) of the vlPFC on one's ability to down-regulate negative affect using cognitive reappraisal. Based on prior correlational evidence, it was hypothesized that inhibition of the vlPFC in humans would disrupt reappraisal-based regulation of negative affect. Here, we examined healthy individuals' subjective rating of their own emotional state after viewing negatively-valenced visual stimuli while they engaged in reappraisal-based emotion regulation. Immediately prior to performing the reappraisal task, participants received a 30-minute low-frequency (1Hz) rTMS of the left vlPFC, right vlPFC, or sham stimulation (left or right vlPFC randomized). All participants received each of the three stimulation-types in a randomized and counterbalanced order in separate sessions. The TMS coil was stereotactically positioned using each participant's T1-weighted structural MRI acquired prior to the stimulation procedure. Inhibition of the left vlPFC decreased the degree to which individuals were able to down-regulate negative affect when compared to sham stimulation ($p = < .05$). By contrast, inhibition of the right vlPFC potentiated the ability to use reappraisal to down-regulate negative affect ($p = < .05$). These data suggest that the vlPFC exerts a causal influence on reappraisal-based emotion regulation, and suggests there may be laterlality-specific influences on this process.

Disclosures: J.U. Kim: None. **D.H. Zald:** None. **S.R. Levine:** None. **L.R. Blair:** None.

Poster

582. **Neural Circuits for Regulating Stress and Emotion**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 582.13/LLL63

Topic: F.03. Motivation and Emotion

Support: ICM P09-015F

ICM P10-001-F

The Guillermo Puelma Foundation

Title: Pharmacological study on the autonomic modulation of the pupillary response during emotional processing

Authors: *S. I. BRUGUÉS SELEME, E. BRUNETTI, M. HERRERA-MARSCHITZ, P. E. MALDONADO;
Univ. De Chile, Santiago, Chile

Abstract: Susana Brugues[1], Enzo Brunetti[1], Mario Herrera-Marschitz[1] & Pedro E. Maldonado[1,2]. BNI[1] & CENEM[2] , Faculty of Medicine, University of Chile, Santiago, Chile. The pupillary response is a valuable physiological marker to study autonomic changes linked to emotional processes, because of its differential and specific responses to visual stimuli with positive or negative emotional valences. However, the particular contributions of the sympathetic and parasympathetic pathways to the dynamics of pupillary response in an emotional context are still unknown. In this study we aimed to examine the consequence of pharmacological inhibition of the sympathetic and parasympathetic components of the pupillary response to emotional stimuli. We measured the pupillary response of 23 healthy individuals after the presentation of 30 images with positive, negative and neutral emotional valences taken from the International Affective Pictures System database. We compared the responses of both pupils of each subject, after instillation of autonomic modulators in one of the eyes. We employed Brimonidine a sympathetic inhibitor (dilator muscle blocker) and Tropicamide, a parasympathetic inhibitor (sphincter muscle blocker), delivered through an ocular topical solution. Both substances evoked marked anisocoria, but the pupil was still responsive to the onset of emotional images. We found differences in pupillary responses to negative versus positive and neutral emotional stimuli early on the pupillary contraction phase of the response. These differences could not be explained by the luminance factor, which was strictly controlled. We also found that the pupillary dilation for negative emotional stimuli was considerably affected by the sympatholytic agent, thereby suggesting that pupillary response modulation to negative stimuli is mainly sympathetic. These

findings show that the processing of emotional stimuli with different valences is linked to specific patterns of autonomic changes that can be detected -with a high temporal resolution- by of dynamical measures in pupil size.
Supported by ICM P09-015F, ICM P10-001-F, and The Guillermo Puelma Foundation.

Disclosures: S.I. Brugués Seleme: None. **E. Brunetti:** None. **M. Herrera-Marschitz:** None. **P.E. Maldonado:** None.

583**Poster**

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.01/LLL64

Topic: F.03. Motivation and Emotion

Support: Emory Egleston Children's Research Center

Children's Healthcare of Atlanta

National Center for Research Resources P51RR165

Title: Incubation of cocaine-induced habits in adolescence

Authors: L. M. DEPOY, *S. L. GOURLEY;
Pediatrics, Neurosci. Program, Emory Univ., Atlanta, GA

Abstract: Adolescence is a period of vulnerability to the development of many psychiatric disorders, including substance dependence disorders that persist across the lifespan. Incubation of certain biological factors associated with addiction may play a causal role. We explored this hypothesis in the context of cocaine-induced stimulus-response habits, which are increasingly considered a causal factor in the development and maintenance of addiction. Here, adolescent or adult mice were exposed to cocaine, and then decision-making strategies were characterized. Mice with a history of subchronic cocaine exposure in adolescence, but not adulthood, developed stimulus-response habits at the expense of engaging in goal-directed decision-making strategies, but only if behavioral characterization occurred following an incubation period. In addition to these behavioral changes, orbitofrontal prefrontal cortex (oPFC) dendritic spines were eliminated and dendrites retracted in adulthood, following adolescent cocaine exposure. The "premature" shift from response-outcome to stimulus-response decision-making strategies was replicated by site-specific infusions of an Abl-family kinase inhibitor STI-571 into the oPFC immediately following response-outcome contingency degradation. Together, these findings suggest that

adolescent cocaine exposure confers behavioral vulnerabilities in adulthood by altering cellular structure during adolescent development. We argue that cytoskeletal reorganization in adolescence is necessary for normative engagement in goal-directed response strategies, and that cocaine may impact lifelong decision-making strategies by interfering with the structural refinement of the neocortex during adolescence.

Disclosures: L.M. DePoy: None. **S.L. Gourley:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.02/LLL65

Topic: F.03. Motivation and Emotion

Title: Forced sedentary home cage conditions decreases cocaine-induced locomotor stimulation in rats

Authors: *M. J. WILL[1], M. MCCABE[2], K. PARKER[2], H. JOHNS[3], M. ROBERTS[3], F. BOOTH[3];
[1]Dept Psych - Life Sci. Cntr, Univ. Missouri-Columbia, COLUMBIA, MO; [2]Psychology, [3]Univ. of Missouri, Columbia, MO

Abstract: The current study examined the interaction of the locomotor response to cocaine in rats that either had 6 week access to a running wheel (voluntary running) exercise or no wheel (forced sedentary) condition. Upon arrival in the laboratory, 16 rats were given either access to a home cage environment with locked (forced sedentary) or free running wheel (voluntary exercise) access for the duration of the experiment. All rats were housed singly and had ad libitum access to home cage chow. Running distance and duration, as well as food intake and body weight, was monitored for 6 weeks. At the end of Week 6, rats were habituated to the locomotor activity apparatus (30x30x45cm) for 70 min on Day 1. All testing was conducted during the middle of the light cycle (1000-1200) and consisted of a 10 pre-injection baseline, followed by a 60 min drug response. All rats were assessed for locomotor activity in response to a low and high dose of cocaine (10mg/kg, or 20mg/kg; i.p.). All rats received alternating low vs. high dose treatments for a total of 10 sessions, including a baseline (0mg/kg) session in between each cocaine session. Each session was separated by at least 2 days. Both groups of rats (voluntary and sedentary) displayed a similar locomotor response to the initial low dose cocaine treatment. However, in response to the initial high dose treatment, the sedentary group displayed

a significantly lower locomotor response compared to the voluntary runner group. This trend continued across subsequent repeated dose sessions.

Disclosures: M.J. Will: None. **M. McCabe:** None. **K. Parker:** None. **H. Johns:** None. **F. Booth:** None. **M. Roberts:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.03/MMM1

Topic: F.03. Motivation and Emotion

Title: Evidence of cross-sensitization between stress and alcohol in rats: Alcohol intake and locomotor behavior in rats exposed to maternal separation or social defeat stress

Authors: *B. E. CALDWELL, E. JACOBS-BRICHFORD;
Ithaca Col., Ithaca, NY

Abstract: The relationship between stress and risk for developing alcohol (ETOH) use disorders has been studied extensively in human and nonhuman animal models. The relationship between stress and ETOH drinking is likely modulated by many factors. Numerous researchers have concluded that individuals are at risk for developing maladaptive coping strategies if they are exposed to early life trauma. Although stress is known to sensitize animals to locomotor effects of stimulant drugs, the literature has not demonstrated evidence for sensitization to alcohol effects in rat populations. The present study examined the effects of maternal separation stress during the postnatal period, followed by social defeat stress in adulthood, on ETOH drinking and locomotor sensitization in male Long Evans rats. Newborn litters of Long Evans rats were culled to 6 pups per litter the day of parturition. Litters were randomly assigned to either maternal separation (MS) or animal facility rearing (AFR). MS began on post-natal day 1 (PND1), continued daily for 14 days, and pups were weaned on PND21. On PND 60, rats were assigned to either social defeat stress (DS) or non-stress groups. DS rats were exposed to 5 daily 5-minute encounters with an aggressive male conspecific. Three weeks following defeat, rats were tested for ETOH preference using a three-bottle choice protocol (10%, 5%, and 0% ETOH, all mixed with 0.5% saccharin). In addition, all animals were tested for locomotor response to an ETOH challenge in an open field apparatus at two time points, and were examined for differences relative to a saline trial and change in sensitivity to ETOH from Time 1 to Time 2. Results showed that maternally separated animals demonstrated distinctly different patterns of ethanol consumption, compared to other groups. MS rats drank less (in g/kg) ETOH during both 24-Hr

and 1-Hr access conditions, demonstrating a consistent preference for the 0% solution. In contrast, the MS/DS (both stressors) group drank the most ETOH, and drank more of the high concentration fluid than any of the other groups. Analyses of locomotor behaviors in the open field showed MS, but not AFR, rats demonstrated an increase in sensitivity to the locomotor-stimulating effects of ETOH from Time 1 to Time 2. These results suggest that neurological changes due to MS may predispose animals to an increased sensitivity to ETOH, but that adult stress is a determining factor in the transition to escalated patterns of alcohol intake.

Disclosures: B.E. Caldwell: None. **E. Jacobs-Brichford:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.04/MMM2

Topic: F.03. Motivation and Emotion

Support: This work was supported by Mid-career Researcher Program through NRF grant funded by the MEST (2011-0016038)

This work was supported by National Research Foundation of Korea (NRF) grant funded by the Korea government [MEST] (No.20120009400)

This work was supported by Korea Institute of Oriental Medicine (K13290)

Title: The involvement of spinal ascending pathways in acupuncture inhibition of cocaine-induced locomotor activity

Authors: *H.-Y. KIM, S. KIM, S. JANG, M. YEO, C. IM, B. LEE, C. YANG;
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Abstract: We have previously demonstrated that acupuncture at Shenmen (HT7) points has powerful inhibitory effects on addictive behaviors of abused drugs including cocaine, alcohol and morphine. Our recent study suggested that HT7 inhibition of cocaine-induced locomotor activity is mediated by A-fiber activation of ulnar nerve originating from superficial and deep tissue. To investigate the possible mechanism of acupuncture on spinal ascending pathways, HT7 inhibition of cocaine-induced hyperactivity were evaluated after surgical transection of dorsal column (DC) or spinothalamic tract (STT) pathway in rats. Furthermore, to identify the involvement of secondary neurons in DC and STT pathways, the acupuncture effects were examined after chemical lesion of ventral posterior lateral nucleus (VPL). Locomotor activity

was measured in male Sprague-Dawley rats treated with lesion using a video tracking system after an intraperitoneal injection of cocaine (15 mg/kg). Acupuncture was applied at bilateral HT7 points for 20 s using a mechanical acupuncture device immediately after systemic cocaine injection. HT7 acupuncture suppressed cocaine-induced locomotor activity, which was blocked by lesion of cuneate nucleus or dorsal column, indicating requirement of DC pathways in traveling acupuncture signals. Additionally, HT7 inhibition of cocaine-induced hyperactivity was prevented by surgical transection of STT pathway or chemical lesion of VPL of both DC and STT pathways. Results suggest that both DC and STT pathways transmitting acupuncture afferent signals may mediate acupuncture's role in suppressing cocaine locomotion.

Disclosures: H. Kim: None. **S. Kim:** None. **S. Jang:** None. **M. Yeo:** None. **C. Im:** None. **B. Lee:** None. **C. Yang:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.05/MMM3

Topic: F.03. Motivation and Emotion

Support: NIH-NCRR (2G12-RR003051)

NIMHD (8G12-MD007600)

Conte Center Grant (P50 MH086400)

MBRS-RISE-MSC (R25-GM061838)

Title: Deep brain stimulation of the ventral striatum impairs extinction of morphine-induced conditioned place preference

Authors: *F. J. MARTINEZ[1], J. RODRÍGUEZ-ROMAGUERA[2], F. H. DO MONTE[2], O. A. MUÑIZ-SEDA[1], G. J. QUIRK[2], J. L. BARRETO-ESTRADA[1];
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Abstract: Deep brain stimulation (DBS) is a neurosurgical procedure used to treat refractory neurological and psychiatric disorders. Recent studies have suggested that DBS of the ventral striatum may be a potential target for treating addictive disorders (Luigjes et al., 2011). We recently showed in rats that DBS of the dorsal portion of ventral striatum (dorsal-VS) reduced fear expression and enhanced fear extinction (Rodríguez-Romaguera et al., 2012). Here, we

examined whether DBS of dorsal-VS could also reduce the expression of morphine-induced conditioned place preference (CPP), and enhance its extinction learning. Male Sprague-Dawley rats were stereotaxically implanted with bipolar electrodes aimed at dorsal-VS (−6.5 mm DV, ±2.0 mm ML, and +1.2 mm AP). Using a two-compartment CPP box, rats were conditioned across 8 days to prefer the side paired with morphine (5 mg/kg, s.c.). Subsequently, rats expressing morphine-CPP received 6 extinction sessions on 6 consecutive days, together with dorsal-VS DBS (130 Hz, 0.1 ms pulse, 100 µA, 60 min) or sham stimulation. DBS did not reduce the expression of morphine-CPP, as indicated by equivalent % time spent in the morphine paired side (Sham: 70%, DBS: 68%). Surprisingly, DBS impaired extinction of CPP, as indicated by a high % of time spent in the morphine paired side throughout extinction (Day 6 - Sham: 52%, DBS: 74%, ANOVA repeated-measures between group; $p<0.05$). Additional experiments showed that the DBS itself did not induce CPP, or alter rats' locomotion. These results suggest that ventral striatum DBS may have opposite effects on fear- vs. reward-extinction. Furthermore, it suggests that the dorsal-VS site may not be a promising target for treating addictive disorders with high-frequency DBS.

Disclosures: F.J. Martinez: None. **J.L. Barreto-Estrada:** None. **O.A. Muñiz-Seda:** None. **J. Rodríguez-Romaguera:** None. **F.H. Do Monte:** None. **G.J. Quirk:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.06/MMM4

Topic: F.03. Motivation and Emotion

Support: Korean Food and Drug Administration

Title: Propofol pretreatment induced place preference and self-administration of the NMDA receptor antagonist-benzodiazepine combination, Zoletil®

Authors: *I. I. DELA PEÑA[1], J. DE LA PEÑA[1], A. MUHAMMAD[1], K. JUNG[1], H. KIM[1], C. SHIN[2], J. CHEONG[1];
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Abstract: In South Korea, there are concerns on the human use/abuse of the NMDA receptor antagonist-benzodiazepine combination, zoletil. Previously, we have shown that zoletil per se has no motivational effects. However, when administered to rats repeatedly pre-exposed to other

psychoactive drugs of abuse (ketamine, diazepam), zoletil produced significant conditioned place preference (CPP) and self-administration (SA). In the present study, we evaluated whether pre-exposure to propofol induce significant CPP and SA towards zoletil. Male Sprauge Dawley rats were repeatedly (14 days) pretreated with propofol (10, 30, or 60 mg/kg), then responses toward zoletil conditioning and self-administration were assessed. The results showed that propofol-pretreated animals spent more time on the zoletil-paired chamber, as compared to the saline-pretreated group, exhibiting significant zoletil CPP. This was supported by the findings in the SA test wherein propofol-pretreated rats facilitated and maintained significant zoletil SA. Taken together, these findings demonstrate that repeated pre-exposure to propofol may subsequently induce reward and reinforcement towards zoletil. It is possible that propofol-users may use zoletil as a substitute drug, probably because propofol is a restricted/controlled drug in South Korea while zoletil isn't. The careful use and monitoring of these psychoactive substances are highly stressed.

Disclosures: I.I. dela Peña: None. **J. de la Peña:** None. **A. Muhammad:** None. **K. Jung:** None. **H. Kim:** None. **C. Shin:** None. **J. Cheong:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.07/MMM5

Topic: F.03. Motivation and Emotion

Support: 5P01DA008227

KAKEN24591735

Title: Functional role of the N-terminal domain of deltaFosB in responses to stress and drugs of abuse

Authors: *Y. N. OHNISHI[1,2], Y. H. OHNISHI[2], V. VIALOU[2], E. MOUZON[2], Q. LAPLANT[2], A. NISHI[1], E. J. NESTLER[2];
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Abstract: Previous work has implicated the transcription factor, ΔFosB, acting in the nucleus accumbens, in mediating the pro-rewarding effects of drugs of abuse such as cocaine as well as in mediating resilience to chronic social stress. However, the transgenic and viral gene transfer models used to establish these ΔFosB phenotypes express, in addition to ΔFosB, an alternative

translation product of ΔFosB mRNA, termed Δ2ΔFosB, which lacks the N-terminal 78 aa present in ΔFosB. To study the question of the possible contribution of Δ2ΔFosB to these drug and stress phenotypes, we prepared a viral vector that overexpresses a point mutant form of ΔFosB which cannot undergo alternative translation as well as a vector that overexpresses Δ2ΔFosB alone. Our results show that the mutant form of ΔFosB, when overexpressed in the nucleus accumbens, reproduces the enhancement of reward and of resilience seen with our earlier models, with no effects seen for Δ2ΔFosB. Overexpression of full length FosB, the other major product of the FosB gene, also has no effect. These findings confirm the unique role of ΔFosB in nucleus accumbens in controlling responses to drugs of abuse and stress.

Disclosures: Y.N. Ohnishi: None. **Y.H. Ohnishi:** None. **V. Vialou:** None. **E. Mouzon:** None. **Q. LaPlant:** None. **A. Nishi:** None. **E.J. Nestler:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.08/MMM6

Topic: F.03. Motivation and Emotion

Support: FWF grants W1206-B18 (JP, KK) and P18787-B05.

Title: Signal processing in neuronal circuits mediating the reorientation from cocaine to social interaction

Authors: *J. M. PRAST, K. K. KUMMER, G. ZERNIG, A. SARIA;
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Abstract: A main challenge in the therapy of drug dependent individuals is to help them reactivate their interest in non-drug-associated activities. Among these activities, social interaction is doubly important because treatment adherence itself depends on it. We developed a rat animal experimental model based on the conditioned place preference (CPP) paradigm in which only four 15-min episodes of social interaction with a gender- and weight-matched male conspecific (i) reversed CPP from cocaine to social interaction despite continuing cocaine training and (ii) inhibited the reinstatement of cocaine CPP (Fritz et al. 2011, Addiction Biology 16:273-284). The reversal of CPP from cocaine to social interaction was enhanced by the sigma1 receptor antagonist BD1047 with an ED50 of 0.0036 mg/kg (i.p.) (Fritz et al. 2011, Pharmacology 87:45-48). Social interaction also reversed cocaine CPP-induced expression of the immediate-early gene zif268 in the nucleus accumbens shell, the central and basolateral amygdala and the ventral tegmental area (Fritz et al. 2011, Addiction Biology 16:273-284).

These findings suggest that social interaction, if offered in a context that is clearly distinct from the previously drug-associated ones, may profoundly decrease the incentive salience of drug-associated contextual stimuli. We propose that different neuron essembles in the nucleus accumbens core (AcbC) and the nucleus accumbens shell (AcbSh) would differentially mediate the CPP for cocaine vs. social interaction (Prast et al. 2012, Pharmacology 90:264-273). We are currently investigating which type of neurons in the AcbC is affected by this reversal.

Disclosures: J.M. Prast: None. **K.K. Kummer:** None. **G. Zernig:** None. **A. Saria:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.09/MMM7

Topic: F.03. Motivation and Emotion

Support: NIH Grant P50-DA026306

Title: Altered reward expectancy in individuals with prior methamphetamine dependence

Authors: *A. BISCHOFF-GRETHE, C. G. CONNOLLY, S. J. JORDAN, G. G. BROWN, M. P. PAULUS, R. K. HEATON, S. P. WOODS, I. GRANT, .. THE TMARC GROUP;
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Abstract: Despite advances in the understanding of the functional brain anatomy of reward expectancy in non-abusing adults, little is known about how these brain substrates might be altered in stimulant abusing individuals. Seventeen abstinent adults with a history of prior methamphetamine dependence (METH+) and twenty-three healthy comparison adults (METH-) performed a probabilistic feedback expectancy task during blood-oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI). Participants were presented with visual cues that were probabilistically associated with monetary gain, loss, or neutral outcomes. Frontal systems measures associated with apathy, impulsivity/disinhibition, and sensation-seeking were acquired at a separate session. METH+ were found to have elevated apathy ($t(20.30)=6.82$, $p<0.001$, $d=2.2$) and impulsivity ($t(28.17)=5.51$, $p<0.001$, $d=1.8$) scores relative to METH-. Region of interest analyses within the left ventral striatum ($F(1,36)=5.27$, $p=0.03$, $d=0.7$) and left anterior caudate ($F(1,36)=8.66$, $p=0.006$, $d=1.0$) revealed group differences for stimuli associated with potential financial outcome. Specifically, post hoc analyses revealed that METH- exhibited a greater response than METH+ within both the ventral striatum ($z=2.3$, $p=0.02$) and the anterior caudate ($z=3.1$, $p=0.002$). In METH+, apathy scores were negatively correlated with the left anterior caudate's percent signal change response for the expectancy of financial gain

(Spearman's rho=-0.69, p=0.002), and sensation seeking was negatively correlated with the percent signal change for the expectancy of financial loss within the same region (Spearman's rho=-0.63, p=0.007). Overall, these findings suggest that the neural response to the potential for financial gains and losses is attenuated in areas associated with reward processing in the METH+ group. This is further supported by the association of greater apathy and sensation seeking scores with reduced striatal response to potential wins and losses, respectively. An impaired ability to evaluate future risks and benefits may underlie the altered decision making seen in METH+ individuals and increase the likelihood of risky behavior.

Disclosures: A. Bischoff-Grethe: None. **C.G. Connolly:** None. **S.J. Jordan:** None. **G.G. Brown:** None. **M.P. Paulus:** None. **R.K. Heaton:** None. **S.P. Woods:** None. **I. Grant:** None. **.. The TMARC Group:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.10/MMM8

Topic: F.03. Motivation and Emotion

Support: DA006886

DA032270

Title: Firing of lateral preoptic area during cocaine self-administration

Authors: *D. J. BARKER, B. S. STRIANO, D. H. ROOT, A. P. PAWLAK, A. T. FABBRICATORE, M. O. WEST;
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Abstract: The Lateral Preoptic area is a GABAergic target of the nucleus accumbens. These projections predominantly originate in the rostral portions of the dorsal and ventral shell, as well and the caudal portions of the dorsomedial shell and core and are believed to be one route by which accumbens signaling might initiate or modulate motivated behaviors. Accordingly, it has been shown that the locomotor behaviors associated with the dopaminergic or opioidergic stimulation of the accumbens can be attenuated by injecting GABA agonists into the lateral preoptic area. Although many studies have correlated firing patterns in the accumbens with drug-seeking behaviors, few studies have focused on the role of the lateral preoptic area during drug self-administration. Importantly, recent anatomical work has shown that the lateral preoptic area sends an important neurotensin projection to the ventral tegmental area as well as GABAergic

projections to the ventral tegmental area, lateral habenula, and rostromedial tegmental nucleus. Thus, the lateral preoptic area is well positioned to influence reward circuitry. Therefore, the goal of the present study was to examine lateral preoptic neurons during cocaine self-administration and determine the neural correlates of lateral preoptic firing to drug-seeking behaviors.

Disclosures: D.J. Barker: None. **B.S. Striano:** None. **D.H. Root:** None. **A.P. Pawlak:** None. **A.T. Fabbricatore:** None. **M.O. West:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.11/MMM9

Topic: F.03. Motivation and Emotion

Support: DA026854

Title: Neurobiological consequences of concurrent chronic stress and nicotine exposure in adult rats previously treated with nicotine during adolescence

Authors: *L. F. ALCANTARA, B. L. WARREN, E. M. PARISE, C. A. BOLAÑOS-GUZMÁN;
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Abstract: There is a high rate of comorbidity between mood disorders and tobacco consumption. Thus, it has been suggested that afflicted individuals may use nicotine to manage their mood. We have demonstrated that nicotine exposure during adolescence, but not adulthood, leads to a depression-like state manifested by decreased sensitivity to natural reward (sucrose preference) and enhanced sensitivity to both stress- and anxiety-eliciting situations in adulthood. Re-exposure to nicotine prior to the forced swim test (FST), a potent stressor, increases the incidence of escape-like behaviors, implying an anti-depressant-like affect. Additionally, chronic nicotine exposure during adolescence increases expression of the extracellular signal-regulated protein kinase 2 (ERK2), a signaling cascade implicated in motivation and mood regulation, and re-exposure to nicotine normalizes altered ERK2 activity within the ventral tegmental area (VTA). This suggests that nicotine exposure in adulthood could reverse the deficits caused by adolescent nicotine exposure, both at the behavioral and biochemical level. However, stress rarely occurs as a singular event and it is not known whether nicotine exposure during adulthood, in rats previously exposed to nicotine during adolescence, would buffer behavioral responses to chronic stress. To assess the possibilities, we exposed adolescent rats to saline or nicotine (0.32

mg/kg) for 15 days (postnatal day 35-49) and then left undisturbed for 4 weeks. At this time, rats were exposed to a chronic mild stress (CMS) paradigm during which half of each treatment group received concurrent saline or nicotine (0.32 mg/kg). Rats were then subjected to the elevated plus maze and the FST at various points throughout the CMS regimen to assess changes in anxiety- and depression-like behaviors, respectively. We also assessed mRNA and protein levels within the VTA, after CMS, to determine how nicotine re-exposure before acute stress differs from nicotine re-exposure during chronic stress. Our findings indicate that nicotine re-exposure in adulthood buffers the negative behavioral consequences induced by CMS exposure. Interestingly, preliminary results suggest that expression of ERK2 in the VTA is also normalized by nicotine re-exposure in adulthood. Together, this work suggests that re-exposure to nicotine in adulthood buffers the negative consequences induced by early life exposure to nicotine.

Disclosures: L.F. Alcantara: None. **B.L. Warren:** None. **E.M. Parise:** None. **C.A. Bolaños-Guzmán:** None.

Poster

583. **Brain Mechanisms Mediating Interactions between Rewards and Drugs**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 583.12/MMM10

Topic: F.03. Motivation and Emotion

Support: NSERC

CIHR

Title: Dissociating the psychoactive effects of distinct marijuana compounds in the mesocorticolimbic circuitry

Authors: *J. ZUNDER, S. R. LAVIOLETTE;
Dept. of Anat. and Cell Biol., The Univ. of Western Ontario, London, ON, Canada

Abstract: The mesocorticolimbic system, composed of the basolaetral amygdala (BLA), medial prefrontal cortex (mPFC), nucleus accumbens (NAcc) and ventral tegmental area (VTA), is responsible for processing the emotional and motivational significance of incoming sensory information. Dysfunctions in the underlying neural systems that regulate these processes may lead to aberrant salience attribution to what would normally be insignificant sensory experiences or events. In the extreme, such attribution may lead to psychotic ideation and persistent maladaptive behaviours.
The mesocorticolimbic system contains high levels of dopamine and cannabinoid CB1 receptors,

both of which have been implicated in the etiological profile of schizophrenia. A growing body of evidence supports the link between heavy marijuana exposure and an increased risk of developing schizphrenia. However, emerging clinical evidence suggests a functional dissociation between the two main pharmacological components of cannabis, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (Δ9-THC). While Δ9-THC may precipitate, or even exacerbate psychotic symptoms, CBD has been shown to have antipsychotic and antianxiety properties comparable to conventional antipsychotic medications. CBD has been shown to be a weak antagonist of the CB1 receptor; in contrast, Δ9-THC acts as a partial CB1 agonist. Our previous work has shown that modulation of CB1 receptors in the mPFC-BLA pathway can mediate the emotional valence of an associative fear memory in a novel olfactory fear-conditioning paradigm. Activation of CB1 receptors in these areas results in the potentiation of an associative fear memory, while blockade of CB1 receptors prevent the formation of this memory. Our current objective is to extend these findings to other areas of the mesocorticolimbic system including the shell of the nucleus accumbens (NA Sh) and to examine the efficacy of the two major marijuana compounds (CBD and Δ9-THC) in mediating emotional learning and memory formation. Preliminary results suggest that CBD has rewarding properties and can block the formation of a fear memory to a normally highly salient footshock (0.8mA). In contrast, Δ9-THC seems to have aversive properties and potentiates the formation of a fear memory to a normally non-salient footshock (0.4mA). Ongoing studies are investigating the effects of chronic juvenile exposure to CBD and Δ9-THC on learning, memory and anxiety, and how these effects may be modulated through interactions with the serotonergic and mesolimbic dopaminergic systems respectively.
(Words: 369)

Disclosures: J. Zunder: None. **S.R. Laviolette:** None.

584**Poster**

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.01/MMM11

Topic: F.04. Neuroethology

Support: HHMI funding

Title: Localization of ultrasonic vocalizations emitted by both male and female mouse models of Fragile X while socially interacting

Authors: *J. P. NEUNUEBEL, A. L. TAYLOR, B. J. ARTHUR, S. E. R. EGNOR;
Howard Hughes Med. Inst. - Janelia Farm Res. Campus, Ashburn, VA

Abstract: Autism is a neural developmental disorder that affects social behavior and impairs communication. Many genetically engineered mouse models of autism have shown deficits in social behavior and the production of ultrasonic vocalizations (Jamain et al., 2008; Rotschafer et al., 2012; Schmeisser et al., 2012). However, previous studies reporting vocal deficits for adult mice in social contexts have been limited by the inability to identify which mouse was vocalizing. Thus, the effect that a mouse's vocal interactions have on its behavior remains unknown. To address this issue, we simultaneously recorded vocalizations and behavior from groups (2 males and 2 females; 6-11 weeks old) of wild type (C57BL/6J; n = 7) or Fragile X (B6.129-Fmr1tm1Rbd/j; n = 7) mice over a period of five hours. Vocalizations were recorded in a large, acoustically favorable cage with an array of four ultrasonic microphones (Avisoft; CM16/CMPA). The source location of each vocalization was determined from the differences in arrival time between each pair of microphones and assigned to one of the four possible sources. Because of this method, our study is the first to identify the source of the vocalizer when multiple mice are present in the same environment. The results showed that both males and females emit ultrasonic vocalizations during social interactions regardless of their genetic background. Furthermore, provisional data suggest that each animal vocalized throughout the entire five-hour cohabitation period. Our future work will focus on quantifying the differences in vocal interaction patterns between wild type and Fragile X mice while examining the behavioral dynamics of the two groups during these periods.

Disclosures: J.P. Neunuebel: None. **A.L. Taylor:** None. **B.J. Arthur:** None. **S.E.R. Egnor:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.02/MMM12

Topic: F.04. Neuroethology

Support: HHMI's Janelia Farm funding

Title: Female rejection and male vocal behavior may play an intimate role in the mating behavior of the house mouse

Authors: *K. SEAGRAVES, J. NEUNUEBEL, R. S. E. EGNOR;
HHMI's Janelia Farm, Ashburn, VA

Abstract: Ultrasonic vocalizations produced by adult male mice are thought to facilitate courtship behavior, either by attracting the female or by promoting her to stay close to the male

after the initial contact. Male mice produce ultrasonic vocalizations when exposed to female urine or a live female, and female mice spend more time near vocalizing males than near males who are mute. Additionally, in playback experiments females show a preference for a speaker emitting male vocalizations compared with a speaker producing background noise, and for the vocalizations of unfamiliar non-kin males over those of familiar sibling males. These results argue that females are able to use cues from the vocalizations to evaluate individual males, an ability that could be used in mate choice. However, it is not known whether male ultrasonic vocalizations affect a female's behavioral receptivity, or vice versa. We believe that the presence or absence of female rejection behavior can be used as a spontaneous measure of female receptivity, and by comparing male vocal behavior with the timing of female rejection events we can more fully understand how these two behaviors affect one another.
We simultaneously recorded audio and video data while adult male and female SWR/J mice were paired together in a 0.6 x 0.6 x 0.6 m cage for 10 minute sessions. We used MoTr (a mouse position tracking system developed in the lab [http://sourceforge.net/projects/motr/]) to identify the location of the mice in each video frame, and an automated program to extract time and frequency data for each ultrasonic vocalization in the audio recording. From these data we identified when ultrasonic vocalizations occurred relative to episodes of female rejection, and analyzed the characteristics of those vocalizations. Preliminary analyses show that significantly fewer vocalizations were recorded when the mice were less than 10 cm apart in sessions where female rejection was high, compared with sessions in which female rejection was low (t-test, $p < 0.001$). However, the number of vocalizations produced when the mice were more than 10 cm apart is not significantly different between the two session types. We will further analyze the data to answer a series of questions about how male vocal behavior and female rejection events affect one another, including: a) Does male vocalization rate change after a female rejection event? b) Is there a difference between the characteristics of ultrasonic vocalizations that occurred before and after female rejection events? c) Are there particular characteristics of male vocal behavior that affect the likelihood of female rejection?

Disclosures: K. Seagraves: None. **J. Neunuebel:** None. **R.S.E. Egnor:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.03/MMM13

Topic: F.04. Neuroethology

Support: 5T32HD007228-32

Title: The Reelin-signaling pathway influences calling behavior: A cross-species approach

Authors: *E. FRALEY, S. A. WHITE, P. E. PHELPS;
UCLA, Los Angeles, CA

Abstract: The Reelin-signaling pathway has been implicated in autism spectrum disorders (ASD) through several lines of evidence including: genetic polymorphisms (SNPs) in *RELN*, cytogenetic abnormalities in autistic brain associated with changes on the long arm of chromosome 7 where *RELN* resides, and lower Reelin protein in the brain and blood of patients diagnosed with ASD relative to controls. New epidemiological evidence points to alterations in the primary intracellular signaling molecule Disabled-1 (Dab1) in a population that was found previously to have no ASD-associated SNPs in *RELN*. This finding underscores the importance of assessing the other components of the Reelin-signaling pathway in ASD etiology. Central features of ASD include impaired social interactions and language problems, symptoms that motivate studies in vocal-learning species such as the zebra finch songbird. We recently found that mRNA for one of the Reelin receptors, very-low density lipoprotein receptor (Vldlr), is regulated by singing behavior within the basal ganglia song control nucleus, area X of adult male zebra finches. Behavioral regulation of other components of the pathway, namely Reelin and Dab1, also were observed in area X and a subset of area X cells exhibited robust Dab1 expression. To identify these Reelin-sensitive cells, we are examining the expression of Reelin-signaling molecules in mouse basal ganglia, for which antigenic markers and mutant lines are available. We found Dab1-positive cells in mouse globus pallidus that resemble those in songbird area X. Although mice are not robust vocal learners, general mechanisms of vocal communication can be studied in this genetically modifiable species. A prior report indicated that, when separated from their dams, Reelin-deficient male mouse pups exhibited significantly fewer ultrasonic isolation calls compared to wild-type littermates at day 7. Now we ask if other components of the pathway, namely apolipoprotein receptor E2 (ApoER2), Vldlr and Dab1, also affect calling behavior by recording mouse pup ultrasonic isolation calls from wild-type, heterozygous and mutant mice of each genotype. Preliminary evidence confirms an age effect with reduced calling at day 14 compared to day 7 in all lines. Dab1-deficient male and female pups appear to call less than wild-type littermates at day 7. Call type analyses for this mutant line indicates that Dab1-deficient mice tend to have simpler, shorter call types compared to wild-type mice. This analysis is aided by our development of an automated clustering analysis for classifying call types by adapting methodology for analyzing birdsong.

Disclosures: E. Fraley: None. **S.A. White:** None. **P.E. Phelps:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.04/MMM14

Topic: F.04. Neuroethology

Support: NSF IOS 0842759

Title: Assessing temporal processing in treefrogs using auditory evoked potentials

Authors: *K. M. SCHRODE[1], M. A. BEE[2];
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Abstract: It can be difficult to communicate acoustically in large social groups due to high noise levels, which can mask signals of interest. The human auditory system can exploit decreases or "dips" in the overall level of background noise to perceive glimpses of pertinent signals. Just as acoustic communication in social groups is not a uniquely human problem, dip listening is not a uniquely human solution. We have previously found that females of one species of treefrog, Cope's gray treefrog (Hyla chrysoscelis), are also able to exploit dips in background noise to recognize communication signals. However, a related species, the green treefrog (Hyla cinerea), appears incapable of doing so. Dip listening requires the auditory system to track fluctuations in the temporal envelope of sounds. We tested the hypothesis that differences in temporal processing between the two treefrog species can account for the apparent differences in dip listening. To test our hypothesis, we assessed temporal processing in gray and green treefrogs via two auditory evoked potential techniques. For one technique, stimuli consisted of two clicks presented with a varying inter-click interval. The shortest inter-click interval capable of eliciting two distinct responses is a measure of temporal integration time. In both species, we observed two distinct responses to clicks with inter-click intervals averaging as low as 3-4 ms. The second technique, the auditory steady-state response (ASSR), measured the ability of the auditory nerve to phase-lock to fluctuations in the envelope of amplitude-modulated tones. Modulation rate transfer functions (MRTFs) were created from the responses of the ASSR. The MRTFs were also very similar both in shape and magnitude for the two species. Our results indicate that temporal processing, at least at the level of the auditory nerve and as assessed by these two techniques, differs little between the two species. We did observe small species differences, but conclude that the differences are probably too small to be biologically relevant. We suspect that there are differences in temporal processing at higher levels of the ascending auditory system that can account for the species-difference in dip listening.

Disclosures: K.M. Schrode: None. **M.A. Bee:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.05/MMM15

Topic: F.04. Neuroethology

Support: Brains and Behavior Fellowship at Georgia State University

Title: The expression of foxp2 in the brain of adult green tree frogs

Authors: *D. SINKIEWICZ, W. WILCZYNSKI;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Vocal behavior is common within the vertebrate lineage. The more primitive extant species that produce vocalizations do so without having to learn them, while some recently derived groups exhibit the ability to shape the vocalizations they produce through learning. Examining the genetic correlates of vocal communication in more primitive vertebrates provides insight into the evolution of vocal behavior and enables us to understand if there are shared genomic pathways for both learned and unlearned vocalizations. *Foxp2* is a gene that is involved in the learning of vocalizations in humans and songbirds. It has also been described developmentally in fish and amphibians, however, there is little known in regard to *foxp2* and vocalizations in these vertebrates. The green tree frog (*Hyla cinerea*) presents a powerful model for studying the genomics of primitive vocalizations because they produce calls without needing to learn them and only the males vocalize. We identified a portion of the *foxp2* transcript for the first time in *H. cinerea* and used that sequence to produce primers for quantitative PCR. In the present study we collected adult male and female green tree frogs in breeding condition. We then measured *foxp2* in brains from the collected animals following bisection into the telencephalon and the midbrain/hindbrain complex, hypothesizing that males would express higher levels when compared to females and that expression would be predominantly in the midbrain/hindbrain complex. We found that, while there is greater expression in the midbrain/hindbrain complex than in the telencephalon ($p = 0.002$), there is no effect of sex ($p = 0.550$) on expression. The regional difference is maintained when considering only males ($p = 0.05$) or only females ($p = 0.005$). These results indicate that the adult green tree frog midbrain/hindbrain expresses the majority of *foxp2* in both males and females. This region of the brain is home to several areas associated with both vocal production and auditory processing in amphibians. This suggests a strong possibility that *foxp2* is implicated in the vocal communication pathway of the green tree frog. This work was supported by a Brains & Behavior fellowship from GSU awarded to DMS.

Disclosures: D. Sinkiewicz: None. **W. Wilczynski:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.06/MMM16

Topic: F.04. Neuroethology

Title: Concatenation of vocal gestures in a rodent model

Authors: *T. RIEDE;
Univ. of Utah, Dept. Biol., Salt Lake City, UT

Abstract: A small motor unit or gesture of vocal production represents an uninterrupted and unique motor pattern resulting in a specific acoustic pattern. The concept of generating and assembling vocal gestures has been demonstrated in songbirds and is well-described in humans. Nonhuman mammals can syntactically arrange calls into bouts, but the motor basis of call and bout production is little understood. Vocal production in mammals requires the fine-tuned interplay of laryngeal and respiratory movements. The simultaneous investigation of laryngeal and respiratory movements can illustrate how laryngeal and respiratory movements are integrated for sound generation. It could also show if and how flexible both movements are in generating vocal behavior. Vocal gestures were studied using laryngeal EMG and subglottal pressure recordings together with sound recordings in awake spontaneously behaving male Sprague-Dawley rats. Many call types of a rat's ultrasound vocal repertoire consisted of single motor gestures, but one call type, the 'composite call', appeared to be assembled from two or more different gestures. The rate of composite calls increased when a male interacts with a female and ranged between 0.2 and 12% in twelve male rats. Subglottal pressure pattern and laryngeal EMG activity during a gesture were not different if that gesture was produced in calls consisting of a single gesture or in composite calls. Furthermore, two different vocal gestures can be variably assembled into composite calls. Most often, the more dynamic gesture (for example, a trill component) was produced last in a composite call. The data support the hypothesis that rats use the concatenation of vocal gestures to generate more complex composite calls. The study cannot answer at which central level (brainstem or forebrain) the integration of the respective motor control is achieved, but it provides an example for this very basic requirement for the evolution of complex vocal systems.

Disclosure.DisclosureBlock:

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.07/MMM17

Topic: F.04. Neuroethology

Support: NSF Grant 1146501

Title: Distinct neural control of vocal phases in frog calls

Authors: *A. YAMAGUCHI, J. C. BARNES;
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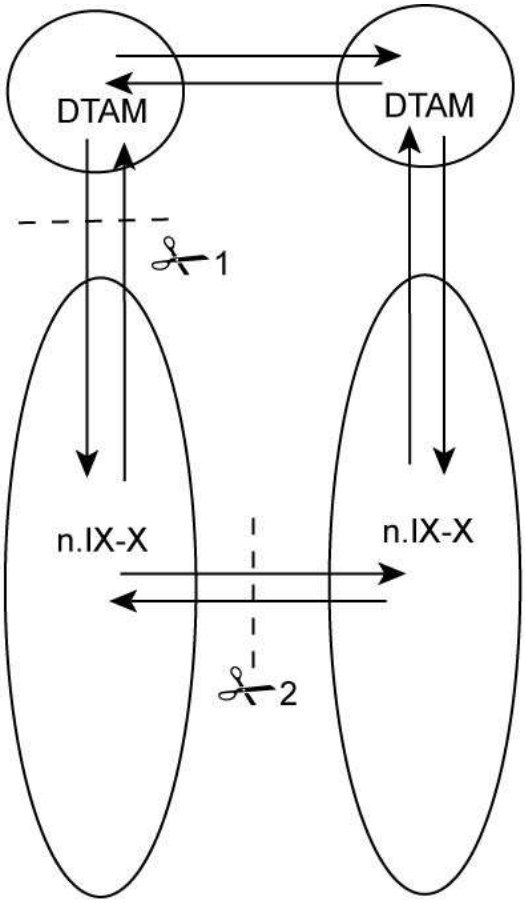
Abstract: The behavior of animals is comprised of a variety of motions. How are the motions underlying a functional behavior generated by the nervous system? In our study, we use the vocalizations of African clawed frogs (*Xenopus laevis*) to address this question. During the breeding season, a male *Xenopus* generates advertisement calls that consist of alternating fast and slow trill phases. Previously, using a fictive preparation *in vitro*, we discovered that the advertisement call is generated by the central pattern generator (CPG) in the brainstem. The vocal CPG consists of a pair of premotor nuclei (DTAM) and a pair of laryngeal motor nuclei (n.IX-X) that are each interconnected (Fig). In this study, we examined how these nuclei contribute differently to the generation of fast and slow trill rhythms. In intact brains, application of serotonin elicits a series of compound action potentials (CAPs) that underlie fast and slow trills from left and right laryngeal motor nerves synchronously. Preliminary results indicate that when DTAM is disconnected from n.IX-X unilaterally (scissors 1), the fast trill deteriorated (in terms of rates and synchrony) while the slow trill remained intact. The results suggest that ascending projection from n.IX-X to DTAM is necessary for the proper generation of fast trill rhythms. In contrast, when connections between the n.IX-X are surgically transected (scissors 2), fast trills remained intact but the slow trills became asynchronous between the two sides, suggesting that slow trill rhythms are generated at the level of n.IX-X. Thus, fast trills seem to be generated by more complex neural mechanisms than slow trills. This may explain why a male *Xenopus* modifies its call structure by increasing the duration of fast trills when it detects a female.



The vocal nuclei that make up the central pattern generator in the brainstem of Xenopus. DTAM and n.IX-X are reciprocally connected.

Disclosures: A. Yamaguchi: None. **J.C. Barnes:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.08/MMM18

Topic: F.04. Neuroethology

Title: Intrasexual analysis of catecholaminergic cell groups and tyrosine hydroxylase fiber innervation of the vocal motor system in a teleost with alternative reproductive tactics

Authors: *Z. N. GHAHRAMANI[1,3], M. TIMOTHY[1], S. KIM[1], G. KAUR[2], P. M. FORLANO[1,3];
[1]Biol., [2]Chem., CUNY Brooklyn Col., Brooklyn, NY; [3]Biol., CUNY Grad. Ctr., New York, NY

Abstract: The plainfin midshipman, Porichtys notatus, is a seasonal breeding marine teleost that produces vocal signals for intraspecific communication. There are two distinct reproductive male morphs: Type I males excavate and defend nests, and vocally court females by rapid contractions of the swim bladder musculature, while type II males do not court females but instead sneak-spawn and steal fertilizations from type I males. Previously established sexual polymorphisms in the hindbrain vocal circuitry of midshipman are related to divergence of male reproductive tactics, explaining the discrepancy in vocalizing ability. Catecholamines, including the neurotransmitters dopamine and noradrenaline, are known regulators of reproduction and sexually motivated behaviors across vertebrates, including vocal-acoustic communication. Therefore, we tested the hypothesis that intrasexual differences in catecholaminergic neuroanatomy may reflect intrasexual differences in vocal circuitry and behavior. Male morphs were collected from nesting sites in Tomales Bay, CA during the summer nesting season and brains were labeled by immunofluorescence for tyrosine hydroxylase (TH-ir), the rate-limiting enzyme in catecholamine synthesis. We compared numbers of catecholaminergic neurons in several nuclei including the periventricular posterior tuberculum, locus coeruleus, vagal and area postrema, known to project, in part, to vocal-acoustic centers. We also quantified catecholaminergic innervation in several nuclei within the descending vocal pathway. Preliminary results suggest no significant differences in the number of TH-ir neurons within analyzed nuclei. However, type II males have a greater density and intensity of TH-ir fibers in the dimorphic vocal motor nucleus, which directly innervates vocal musculature. Our findings support the hypothesis that TH-ir dimorphisms in vocal pathways are substrates of behavioral divergence between the two types of male midshipman, indicating possible mechanisms of neural plasticity modulating social behavior.

Disclosures: Z.N. Ghahramani: None. **M. Timothy:** None. **S. Kim:** None. **G. Kaur:** None. **P.M. Forlano:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.09/MMM19

Topic: F.04. Neuroethology

Support: University of Washington Royalty Research Fund Grant

Title: Estrogen-dependent changes in saccular hair cell density in a vocal teleost fish

Authors: *R. A. MOHR[1], A. B. COFFIN[4,5], M. A. MIDDLETON[7,2], P. SWANSON[7,6], J. A. SISNEROS[1,3];
[1]Dept. of Psychology, [2]Sch. of Aquatic and Fisheries Sci., [3]V.M. Bloedel Hearing Res. Ctr., Univ. of Washington, Seattle, WA; [4]Col. of Arts and Sci., [5]Dept. of Vet. and Comparative Anatomy, Pharmacol. and Physiol., Washington State Univ., Vancouver, WA; [6]Ctr. for Reproductive Biol., Washington State Univ., Pullman, WA; [7]Northwest Fisheries Sci. Center, Natl. Marine Fisheries Service, Natl. Oceanic and Atmospheric Admin., Seattle, WA

Abstract: The plainfin midshipman, Porichthys notatus, is a Pacific Northwest deep-water fish that makes seasonal migrations into the shallow intertidal zone to breed. Parental, or type I, males excavate nest sites under rocky shelters from which they produce low frequency advertisement calls (or "hums") to attract females to mate. Females detect these courtship calls using the saccule, the primary hearing organ in midshipman and most other fish species. Females undergo seasonal shifts in auditory sensitivity, particularly in the frequency range associated with the male hum. These physiological changes have been attributed to fluctuating levels of steroid hormones, including estrogen, and have been observed in the peripheral auditory system at the level of the saccular hair cells and VIIth nerve saccular afferents. Previous work has also shown a seasonal addition of hair cells in the saccule, which are thought to play a role in the increased auditory sensitivity of reproductive females. This study tests the hypothesis that elevated estrogen levels can induce an increase in saccular hair cell density. Non-reproductive female midshipman fish were gonadectomized and implanted with estrogen or control (empty) capsules. After 28-31 days fish were sacrificed and their saccular epithelia were stained with phalloidin (actin-binding hair cell marker), mounted and imaged with confocal microscopy. Hair cell counts were conducted in seven distinct regions across the saccular epithelium. Blood samples were also taken to measure circulating levels of estrogen in the control and implanted fish. Results show an estrogen-dependent increase in hair cell density consistent with our previous work in naturally occurring reproductive fish, suggesting a possible steroid-dependent mechanism for seasonal differences in hearing sensitivity and hair cell plasticity observed in this species.

Disclosures: R.A. Mohr: None. **A.B. Coffin:** None. **J.A. Sisneros:** None. **M.A. Middleton:** None. **P. Swanson:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.10/MMM20

Topic: F.04. Neuroethology

Support: NIH Grant SC2DA034996

Title: Immunohistochemical localization of GABA and serotonin provides an emerging picture of neuromodulator interactivity in a vocal teleost

Authors: *M. TIMOTHY, P. M. FORLANO;
Biol., Brooklyn College, City Univ. of New York (CUNY), Brooklyn, NY

Abstract: The inhibitory neurotransmitter gamma-amino-butyric acid (GABA) is essential for vertebrate neuronal signaling. Moreover, GABAergic neurons are integral components of neuromodulatory networks such as the monoamine serotonergic system. We utilized fluorescent immunohistochemistry against GABA and serotonin to map the putative interaction of these systems in the plainfin midshipman fish, a teleost model of vocal and auditory behavior. Prior anatomical research has shown the presence of monoamine immunoreactive cells and fibers in evolutionarily conserved circuitry involved in the processing and integration of audition and vocalization. Here, we identify GABA signal in cells and fibers throughout these behaviorally relevant nuclei, often in proximity to serotonin-ir signal. GABA-ir cells were found interspersed with serotonergic cells in the superior raphe nuclei. GABA-ir cells and processes were found proximal to the hindbrain-spinal cord vocal pattern generator, isthmal vocal processing areas, and the midbrain periaqueductal gray as well as in auditory nuclei in the hindbrain and torus semicircularis. 5-HT-ir fibers overlapping GABA-ir cells and processes were also identified in vocal-acoustic integration areas throughout the hypothalamus, including the anterior tuberal nucleus and preoptic area. These data suggest a crucial role for GABA as an interneuronal mediator of serotonin signaling in circuitry underlying vocal-acoustic behavior.

Disclosures: M. Timothy: None. **P.M. Forlano:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.11/MMM21

Topic: F.04. Neuroethology

Support: NIH Director’s Pioneer Award

HHMI

Title: Inferred organization of a dinosaur brain

Authors: *C.-C. CHEN[1], K. WADA[3], M. V. RIVAS[4], E. JARVIS[2], D. SOARES[5], D. FRIEDEBERG[2], T. GLENN[6], E. D. JARVIS[2];
[1]Neurogiology, [2]Neurobio., Duke Univ., Durham, NC; [3]Biol. Sci., Hokkaido Univ., Sapporo, Japan; [4]Durham Veteran's Affairs Med. Ctr., Durham, NC; [5]Biol., Univ. of Maryland, College Park, MD; [6]Envrn. Hlth. Sci., Univ. of Georgia, Athens, GA

Abstract: Because dinosaurs are extinct, no known viable brain material exists. The closest living relatives are crocodiles, which pre-date many dinosaurs, and birds, which post-date them. Therefore, the similarities between the brains of crocodiles and birds would suggest shared organization with dinosaur brains. Here, we used thirteen genes [PPAPDC1A, SEMA6A, FOXP1, FOXP2, SLIT1, COUP-TF2, ER81, LHX9, GRIN2D, GRIN2A, ROR-β, DLX6, LHX8] that we found define seven major cerebral subdivisions of the avian brain, consistent with a new understanding of avian brain organization (Jarvis et al., 2013; Chen et al., 2013), to decipher whether some or most of these regions exists in crocodilian brain. We found six regions in the alligator brain with a similar, but not identical organization as the avian brain. Two of these, the striatum and pallidum, make up the basal ganglia, which we know to be conserved among amniotes. The others include the pallial regions that in birds were recently redefined as the arcopallium, nidopallium, mesopallium, hyperpallium and associated primary sensory pallial fields. These regions in birds contain pathways for vocal learning behavior and other complex behaviors, and like in birds show hearing-induced gene expression when hearing alligator vocalizations. The more highly developed subdivision in birds was the hyperpallium, at the dorsal surface of the brain, which contains one of two visual pathways and somatosensory processing areas. Overall, the molecular topographic organization of the crocodilian cerebrum is about 90% similar to that of birds, including the presence of a relatively large pallium, an analogue of the mammalian cortex. These findings suggest that the brains of dinosaurs must have included a cerebrum with these six subdivisions that have the capacity to process complex, cognitive behaviors.

Disclosures: C. Chen: None. **K. Wada:** None. **M.V. Rivas:** None. **E. Jarvis:** None. **D. Soares:** None. **D. Friedeberg:** None. **T. Glenn:** None. **E.D. Jarvis:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.12/MMM22

Topic: F.04. Neuroethology

Title: Defining a new terminology for non-avian reptile brains

Authors: *D. FRIEDEBERG, C.-C. CHEN, E. JARVIS, M. LEAL;
Duke, Durham, NC

Abstract: In 2004 the Avian Brain Nomenclature Consortium published a new avian brain terminology that better reflects our current understanding of the homologies between mammals and birds. This revision was based on neural connectivity, developmental, neurobehavioral, and particularly comparative gene expression profile studies. The consortium concluded that the avian telencephalon is organized into pallial, striatal and pallidal regions that are homologous in all vertebrates. The new nomenclature has replaced the classic system in all contemporary avian neurobiology studies. Though this modern avian nomenclature should by extension include all reptiles, non-avian reptilian studies continue to use a partly obsolete system. This is due in part to the lack of a systematic comparative identification of the telenchepalic regions between birds and non-avian reptiles. In our companion abstract, we discovered that most telencephalic cell populations identified with gene expression markers in the avian brain are also present in their closest living relatives, crocodiles. Here, we used multiple gene expression patterns to delineate the telencephalic subdivisions in two other reptile lineages, lizards and turtles. We found that these markers labeled in the classically defined lizard and turtle dorsal ventricular ridge (DVR) a mesopallium-like region, a nidoplallium-like region, and an arcopallium-like region. These regions had continuous domains that wrapped dorsal to the lateral ventrical, where the hyperpallium and dorsal mesopallium are located in birds. However the relative shapes of these regions were more divergent than the alligator was to birds. We are currently examining additional markers, including those of the primary sensory populations of the pallium. Overall, putative homologues of the revised cell population can be found in non-avian reptiles, but there is divergence on the overall topology of the populations.

Disclosures: D. Friedeberg: None. **C. Chen:** None. **E. Jarvis:** None. **M. Leal:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.13/MMM23

Topic: F.04. Neuroethology

Support: German Research Foundation Grant BO 2471/3-2

LOEWE Grant (German State of Hesse) LingBas

Title: Reconceptualizing the auditory dorsal stream within a unified neurobiological model of audition and language

Authors: *I. BORNKESSEL-SCHLESEWSKY[1], J. P. RAUSCHECKER[2], M. SCHLESEWSKY[3], S. L. SMALL[4];
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Abstract: Successful models of human brain systems are typically based on detailed animal models. However, this approach was long deemed impossible for higher cognitive functions such as language since these, almost by definition, are not shared across species. Within the now generally accepted framework of dual (i.e. dorsal and ventral) auditory pathways, this issue has been debated particularly for the dorsal stream, with several influential dual-stream models of speech and language based on the assumption of a dissociation from the auditory dorsal stream in non-human primates [1,2].
Here, we present a reconceptualization of the auditory dorsal stream as the basis for a new, unified, cross-species model of auditory cognition. Specifically, we posit that the dorsal stream shows a parallel organization to the ventral stream in that it is structured hierarchically and subserves the processing of auditory input from primary auditory regions to posterior temporal, inferior parietal and frontal regions. The crucial difference between the two streams lies in the nature of the computations performed: in contrast to the ventral stream's identification and encoding of successively more complex auditory objects (via experience-based statistical associations) [3], the dorsal stream performs time-dependent computations predicting future outcomes that are subsequently matched against the actual input in the sense of a forward model. This computational characterization applies to auditory spatial information in both monkeys and humans, as well as to speech and other human cognitive tasks that require complex sequence processing (e.g. understanding sentences) [4]. A characterization of the dorsal stream along these lines paves the way for the assumption that both streams operate in monkeys as well as humans and, most importantly, that they process all types of auditory stimuli - from simple sounds to higher-level sequences (e.g. language, music).
In addition to presenting the basic assumptions of this new model, we review recent evidence in its favor, including findings on sentence processing in normal and patient populations as well as the observation of a cortical hierarchy of temporal receptive windows [5].
[1]Hickok, G., & Poeppel, D. (2007). Nat Rev Neurosci, 8, 393-402.
[2]Friederici, A. D. (2012). Trends Cogn Sci, 16, 262-268.
[3]Rauschecker, J. P., & Scott, S. K. (2009). Nat Neurosci, 12, 718-724.
[4]Bornkessel-Schlesewsky, I., & Schlesewsky, M. (2013). Brain Lang, 125, 60-76.
[5]Lerner, Y. et al. (2011). J Neurosci, 31, 2906-2915.

Disclosures: I. Bornkessel-Schlesewsky: None. **J.P. Rauschecker:** None. **M. Schlesewsky:** None. **S.L. Small:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.14/MMM24

Topic: F.04. Neuroethology

Support: NSF GRFP

NIH Grant NS23684

Title: Generation of unique vocalizations via laryngeal filtering and premotor patterning

Authors: *C. L. BARKAN[1], D. B. KELLEY[2];
[1]Neurobio. & Behavior, [2]Biol. Sci., Columbia Univ., New York, NY

Abstract: Xenopus males produce temporally distinct calls that attract female mates and silence male rivals. The X. laevis clade includes 6 geographically distinct populations. Two of these, X. laevis South Africa (SA) and X. laevis Congo (Cg), produce a call composed of two distinct click rate segments. However, in X. laevis SA the segments are fast and slow trills, while in X. laevis Cg the segments are slow trills and single clicks. Segment durations in X. laevis Cg are shorter than in SA. We are using these two populations to determine vocal circuit properties that underlie generation of temporally distinct motor patterns.

Sound production in X. laevis relies on contractions of a single set of muscles in the larynx. Activity recorded from the motor nerve innervating the laryngeal muscles matches the temporal pattern of the click trains comprising vocalizations. The simplicity of this sound production mechanism led to development of two experimentally reduced preparations: an isolated brain that facilitates analysis of the hindbrain vocal central pattern generator (CPG) and an isolated larynx that assesses how CPG output is translated to vocal clicks. An isolated X. laevis SA brain bathed in serotonin produces compound action potentials (CAPs) on the laryngeal nerve that precisely match the temporal pattern of clicks comprising vocalizations. Cells in the pre-motor dorsal tegmental area of the medulla (DTAM) drive the vocal motor neurons that synchronously produce the CAPs and set CAP rate and segment duration. In the isolated X. laevis SA larynx, each stimulus applied to the laryngeal nerves elicits a vocal click.

In X. laevis Cg, we find that CAPs recorded from the laryngeal nerve in response to serotonin match the vocal patterns only during the slow segment, not the single click segment. During the portion of fictive calling corresponding to the single click segment, the nerve produces bursts of fast rate CAPs. Recordings of laryngeal muscle EMG, tension transients and sound production suggest that the larynx transforms trains of fast rate CAPs on the nerve into single vocal clicks,

but faithfully reproduces CAPs at a slow rate. Extracellular recordings indicate that DTAM activity precedes CAP patterns on the nerve and sets the shorter duration of the segments. These findings suggest that similar CPG mechanisms set distinct call segment durations in X. laevis SA and Cg, but that click number is regulated at fast rates by a laryngeal filter in X. laevis Cg and not X. laevis SA. Further investigation of how vocal patterns are created by hindbrain circuitry and filtered by the larynx will provide insight into how each Xenopus population produces a unique call.

Disclosures: C.L. Barkan: None. **D.B. Kelley:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.15/MMM25

Topic: F.04. Neuroethology

Support: NSF grant IOS 0843735

Grass Foundation

Marsden FastStart from the Royal Society of New Zealand

Title: Anterior lateral line nerve encoding to tones and play back vocalisations in free swimming oyster toadfish, Opsanus tau

Authors: C. A. RADFORD[1,2], *A. F. MENSINGER[3,2];
[1]Univ. of Aukland, Aukland, New Zealand; [2]Marine Biol. Lab., Woods Hole, MA; [3]Univ. of Minnesota Duluth, DULUTH, MN

Abstract: In the underwater environment, sound propagates both as a pressure wave and as particle motion, with particle motions dominating close to the source. At the receptor level, both the fish ear and the neuromast hair cells act as displacement detectors and both are potentially stimulated by the particle motion component of sound sources. Here we examined the encoding of the anterior lateral line nerve to sound stimuli in a freely behaving oyster toadfish, Opsanus tau, using the chronic recording technique. Nerve sensitivity and directional responses were constructed using spike rate and vector strength analysis, a measure of phase-locking of spike times to the stimulus waveform. All units showed greatest sensitivity to 100 Hz. While sensitivity did not change with orientation to the stimulus their ability to phase-lock did. Three different types of units were classified, Type 1 (tonic), Type 2 (phasic) and Type 3 (inhibitory).

The Type 1 fibers could be classified further into two sub-types based on their frequency response (Type 1-1 and Type 1-2), which was hypothesised to be related to canal (Type 1-1) and superficial (Type 1-2) neuromast innervation. Oyster toadfish also exhibited sensitivity and phase locked to boatwhistle vocalisations, with greatest spike rates exhibited at the onset of the call. These results provide the first direct evidence that oyster toadfish can use their lateral line to detect behaviourally relevant sound stimuli, which could provide a second sensory pathway to aid in sound source localisation.

Disclosures: C.A. Radford: None. **A.F. Mensinger:** None.

Poster

584. **Vocal Communication: Non-Avian**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 584.16/MMM26

Topic: F.04. Neuroethology

Support: NIH Grant DC-8578

Title: Evidence of voluntary vocal control by the common marmosets (Callithrix jacchus)

Authors: *L. ZHAO, S. ROY, X. WANG;
Dept. of Biomed. Engin., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Humans are capable of exquisite voluntary vocal control in such behaviors as speaking and singing. When presented with altered auditory feedback, human subjects quickly adjust parameters of vocal production to compensate for feedback perturbations. However, it has long been believed that non-human primates are incapable of voluntary vocal control. Instead their vocalizations are stereotyped and do not exhibit compensations when presented with feedback perturbations. Recent experiments in our laboratory in the common marmoset (Callithrix jacchus), a highly vocal New World primate, showed that they exhibited the Lombard effect in noisy background and were capable of adjusting the timing of calls during vocal exchanges in the presence of interfering noise. In the present study, we investigated whether marmosets are capable of altering acoustic structures of their vocalizations when presented with interrupting noises. Marmosets produce long duration calls known as phee calls and are often engaged in antiphonal calling when visual contact is occluded. Our study was based on phee calls and the antiphonal calling behavior paradigm. We introduced acoustic perturbations by playing interfering noises during a vocalization while a marmoset was producing spontaneous phees or engaged in antiphonal calling. The acoustic perturbations led to vocal alterations not only in call timing but also in its spectral characteristics. Our results showed that: (1) with

acoustic perturbations, marmosets produced phee calls with fewer phrases than observed during vocal exchanges in normal conditions; (2) the perturbations resulted in changes in the fine structure of calls (e.g., change in frequency slope was found to be higher in calls with perturbation compared to calls in silent background); (3) when presented with band-pass noise at frequency either below or above the fundamental frequency (F0) of vocalization, marmosets tended to shift their vocalization F0 away from the noise spectrum. Collectively, these results suggest that marmosets rely on auditory feedback to maintain their vocalizations and exhibit voluntary control of the acoustic structures of their vocalizations. [This research is supported by NIH grant DC-8578.]

Disclosures: L. Zhao: None. **S. Roy:** None. **X. Wang:** None.

585**Poster**

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.01/MMM27

Topic: G.04. Physiological Methods

Title: Characterizing human ion channels in induced pluripotent stem cell derived neurons

Authors: *S. STOELZLE, A. HAYTHORNTHWAITE;
Nanion Technologies, Munich, Germany

Abstract: Neurons derived from human induced pluripotent stem cells were characterized using manual and automated patch clamp recordings. These cells expressed voltage-gated Na, Ca and K channels as expected from excitable cells. The Na current was TTX sensitive. About 50% of the Ca current was blocked by 10 μM of the L-type channel blocker nifedipine. Two populations of K channel were present in different proportions: an inactivating (A-type) and a non-inactivating type. The A-type current was sensitive to 4-AP and TEA. Application of γ-aminobutyric acid (GABA) activated a current sensitive to the GABAA receptor antagonist bicuculline. In both devices, comparable action potentials were generated in current clamp. With unbiased, automated patch clamp, about 40% of the cells expressed Na currents, while visual guidance in manual patch clamp provides almost 100% success rate of patching 'excitable cells'. These results show high potential for pluripotent stem cell-derived neurons as a useful model for drug discovery, in combination with automated patch clamp recordings for high throughput and high quality drug assessments at human neuronal ion channels in their correct cellular background.

Disclosures: S. Stoelzle: None. **A. Haythornthwaite:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.02/MMM28

Topic: G.04. Physiological Methods

Support: DGAPA-PAPITT-UNAM 209209

Title: Development of an impedance measurement system to detect temperature changes in single ionic channels using Patch-Clamp technique

Authors: *J. AGUILAR[1], L. ISLAS[2], D. ELIAS-VIÑAS[1];
[1]Bioelectronics seccion, CINVESTAV-IPN, Mexico City, Mexico; [2]Fisiologia, Univ. Nacional Autónoma de México, Facultad de Medicina, Mexico city, Mexico

Abstract: Temperature gating studies of ion channels by patch clamp recording methods have been made difficult by a lack of methods for rapid alteration and detection of temperature in live cells. To address this issue, we developed a closed-loop temperature control system with infrared diode lasers (as heat generator), a cooling system based on peltier cells (thermoelectric cells) controlled by a pulse width modulator (PWM), a digital ohmmeter and a containment chamber. The aim of this system was to obtain a controlled temperature pulse and to detect cell responses induced by temperature changes. The digital ohmmeter measures the bath´s impedance near a cell or membrane path (as a temperature indicator) through electrodes, digitally selects the corresponding gain for the value interval that will be measured in the cell, therefore obtaining a corresponding value to that measurement in ohms. The impedance measurement system has been evaluated through electric simulators and our results corroborate system functionality. The cooling system rapidly diminishes the temperature near a cell, does not interfere with patch clamp recordings and does not heat the containment chamber. Our system has the advantage of been a low cost device and to be a useful tool for recording electrical signals from channels of living cells with an adequate temporal resolution through patch clamp techniques and under precise temperature control.

Disclosures: J. Aguilar: None. **L. Islas:** None. **D. Elias-Viñas:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.03/MMM29

Topic: G.04. Physiological Methods

Title: Transforming the TRP channel drug discovery using medium throughput electrophysiological assays

Authors: J.-M. CHAMBARD, E. TAGAT, P. BOUDEAU, *M. PARTISETI;
Lead Identification Technologies, Sanofi R&D, Vitry sur Seine, France

Abstract: Since the cloning of its first member in 1998, transient receptor potential (TRP) cation channels have become one of the most studied ion channel families in drug discovery. These channels, almost all calcium permeant, have been involved in many different (patho)-physiological and therapeutic areas as diverse as pain, neurodegenerative, cardiovascular, inflammatory diseases and cancer.
At the same time, implementation of automated electrophysiology screening platforms has significantly increased the tractability of ion channels, mainly voltage-gated, as drug targets. The work presented in this poster shows the design and validation of TRP screening assays using the IonWorks Quattro platform (Molecular Devices) allowing a significant increase in throughput to support drug discovery programs. This new player has a direct impact on resources and timelines by prioritizing potential candidates and reducing the number of molecules requiring final testing by manual patch-clamp which is still today the gold standard technology for this challenging drug target class.

Disclosures: J. Chambard: None. **M. Partiseti:** None. **P. Boudeau:** None. **E. Tagat:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.04/MMM30

Topic: G.04. Physiological Methods

Title: A method for patch-clamp recordings of fast-acting ion channels in rat dorsal root ganglion cells

Authors: *J. SVENSSON DALÉN, A. KARLSSON, M. KARLSSON, S. ASPENGREN, P. KARILA;
Cellectricon AB, Mölndal, Sweden

Abstract: We have developed a patch clamp based assay for characterization of fast-acting ion channels in primary dorsal root ganglion (DRG) neurons. This assay is based on the use of primary DRG neurons in culture as a cell model for chronic pain. These neurons retain their sensory functionality and remain responsive to thermal, mechanical and functional stimuli, and when supplemented with nerve growth factor (NGF) they can be used to mimic peripheral sensitization.
The assay utilizes a microfluidic perfusion system, Dynaflow Resolve, to facilitate a stable recording situation and fast and programmable solution exchange. At the start of the experiment, compounds and buffer are loaded in the 16 wells of the Dynaflow Resolve chip. Micro-channels connect each well to a recording chamber where the cells are added. To be able to provide fast solution exchange the DRG neuron is lifted using the patch pipette and positioned in front of the micro-channel outlets. Then, the cell is scanned through the discrete flow zones formed outside the channels.
Data will be presented on the characterization on P2X receptor-mediated ionic currents in dorsal root ganglion neurons.

Disclosures: J. Svensson Dalén: A. Employment/Salary (full or part-time):; Cellectricon AB. **A. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB. **S. Aspengren:** A. Employment/Salary (full or part-time):; Cellectricon AB. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon AB.

Poster

585. New Tools for Studying Channels, Receptors and Single Neurons

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.05/MMM31

Topic: G.04. Physiological Methods

Title: Achieving Quality and Throughput in human nAChR α7 Screening: A Cross-platform comparison study using a recombinant cell model

Authors: *H. WEI, C. W. BENJAMIN, D. H. WERTH;
EMD Millipore Corporation, Discovery & Develop. Solutions, Saint Charles, MO

Abstract: The α7 subtype of nicotinic acetylcholine receptors (nAChRs) is a ligand gated ion channel of broad distribution whose activity can be modulated by endogenous neurotransmitters as well as synthetic ligands. There is a growing body of evidence that nAChR α7 is an important target for Alzheimer's disease, Schizophrenia and other psychiatric disorders. Selective nAChR

α7 agonists, as well as allosteric modulators and pharmaceutically relevant antagonists are in development for the treatment of these diseases. However, the screening efforts for compounds targeting nAChR α7 are constrained due to the unique properties of nAChR α7 such as the low probability of channel opening and rapid desensitization. In addition, the challenges in recording nAChR α7 ion flux are complicated by the need for higher throughput platforms. In this study, we compared the activation profile of the PrecisION™ hnAChR α7/ric3-HEK recombinant cell line (EMD Millipore, Cat. No.: CYL3097) to the endogenous ligand acetylcholine (ACh), reference agonists, antagonists and positive allosteric modulators (PAMs), using three unique recording platforms with a range of throughput capacity: OctaFlow ^{TM} fast perfusion manual electrophysiology system, PatchXpress 7000A and FLIPR$^{TETRA\ TM}$ system. These methods produced consistent pharmacology data within instrumentation, suggesting that the recombinant cell model provides robust data in both low and high-throughput platforms. This study provides validation of the recombinant cell model using different platforms and supports their use for screening nAChR α7 compounds for agonist, antagonist and modulator activity.

Disclosures: H. Wei: None. **C.W. Benjamin:** None. **D.H. Werth:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.06/MMM32

Topic: G.04. Physiological Methods

Support: American Heart Association, Grant number 12IRG9070003

Title: Lab-on-a-chip microfluidic device for kinetically resolved electrochemical detection of adrenal catecholamine secretion

Authors: *K. P. CURRIE[1], I. GES[2], R. L. BRINDLEY[1], F. J. BAUDENBACHER[2];
[1]Anesthesiol & Pharmacol, Vanderbilt Univ. Sch. of Med., NASHVILLE, TN; [2]Biomed. Engin., Vanderbilt Univ., Nashville, TN

Abstract: Release of neurotransmitters and hormones by calcium-regulated exocytosis is a fundamental cellular/molecular process that is disrupted in a variety of psychiatric, neurological, and endocrine disorders. Therefore, this area represents a relevant target for drug and therapeutic development, efforts that will be aided by novel analytical tools and devices that provide mechanistically rich data with increased throughput. Here we outline a microfluidic platform designed to analyze catecholamine secretion from small populations of adrenal chromaffin cells, an important neuroendocrine component of the sympathetic nervous system and versatile

neurosecretory model. Our goal was to develop a modular platform that will: 1) enable rapid and reliable assessment of drug candidates or other manipulations on neuroendocrine secretion (e.g. use cells isolated from transgenic mice, or other rodent models of disease); 2) maintain and enable spatiotemporal analyses of autocrine/paracrine communication within small clusters of chromaffin cells; 3) develop a "sympathoadrenal module" for future "on-chip" integrative analyses of physiological systems.
The devices were fabricated by replica molding using a patterned photoresist on a silicon wafer as the master, and polydimethylsiloxane (PDMS) as the biocompatible polymer. Three microfluidic inlet channels lead to an array of "cell traps", each capable of immobilizing single or small clusters of chromaffin cells. This is assembled onto a glass coverslip with patterned thin film platinum electrodes for electrochemical detection of catecholamines. The response of the platinum electrodes to perfusion of the device with epinephrine / norepinephrine (no cells present) was stable and linearly related to catecholamine concentration. We optimized the cell isolation procedure to enable reliable loading of the device with small populations of bovine chromaffin cells. Robust catecholamine secretion was evoked by perfusion with physiologically relevant secretagogues (direct depolarization with KCl, the cholinergic agonist carbachol, or the neuropeptide PACAP). The response to carbachol was concentration-dependent, and abolished in the absence of extracellular calcium. We also show that the kinetics of catecholamine secretion can be followed with high temporal resolution, and remain stable during multiple rounds of stimulation. Overall, the data we present demonstrate the utility of this microfluidic device for quantitative analyses of the amount and time-course of catecholamine secretion from neuroendocrine chromaffin cells.

Disclosures: K.P. Currie: None. **I. Ges:** None. **R.L. Brindley:** None. **F.J. Baudenbacher:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.07/MMM33

Topic: G.04. Physiological Methods

Support: BINA scholarship for M.M.

Title: Enhancement of neurite outgrowth and neuronal differentiation by using β-NGF conjugated to maghemite nanoparticles

Authors: M. MARCUS[1], H. SKAAT[2], S. MARGEL[2], *O. SHEFI[1];
[1]Fac. of Engin. and Inst. of Nanotechnologies and Advanced Materials, Ramat Gan, Israel;
[2]Dept. of Chem. and Inst. of Nanotechnologies and Advanced Materials, Bar Ilan Univ., Ramat Gan, Israel

Abstract: The ability to increase neuronal differentiation and neuronal growth has great implications in the tissue engineering field. In the present study, we describe a new approach to promote neuronal differentiation and growth, by using a NGF-nano-based tool. Growth factors are critical components in nerve tissue development and repair. The nerve growth factor (β-NGF), a prototypical growth factor, functions as a signaling molecule and stimulates the growth, maintenance and survival of certain target neurons. We found that covalent conjugation of β-NGF to maghemite (γ-Fe2O3) nanoparticles enhances neuronal differentiation in PC12 cells. PC12 cells, a common model for primary neuronal cells, undergo cellular changes when exposed to β-NGF in vitro, i.e., cease proliferation, grow long neurites, and show changes in cellular composition associated with neuronal differentiation. In our study, PC12 cells were exposed to β-NGF, conjugated to maghemite nanoparticles or as a free factor, and the effect on cell-differentiation properties was observed. We found that the stabilization of β-NGF by covalent conjugation to the maghemite nanoparticles significantly enhances PC12 neurites outgrowth, compared to free β-NGF at the same concentration. We also found an increase in the complexity of the branching tree and in the clustering behavior. In addition, neuronal differentiation markers were upregulated in PC12 cells when treated with the conjugated factor. Several concentrations of nanoparticles were tested indicating the effect to be more significant at lower concentrations. Single cell imaging revealed particles accumulation in the soma (but not in the nucleus), in the growth cones and at branching points. We suggest that by covalently binding β-NGF to nanoparticles, β-NGF's half-life is extended and therefore leads to an increase in PC12 differentiation. Our study opens new directions in neuronal repair.

Disclosures: M. Marcus: None. **H. Skaat:** None. **S. Margel:** None. **O. Shefi:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.08/MMM34

Topic: G.04. Physiological Methods

Title: Use of stem cell-derived human neurons to screen a chemical library for potential neurotoxicity

Authors: *M. ROACH[1], R. MALAVARCA[2], K. GOMES[2];
[1]PhoenixSongs Biologicals, Inc., Branford, CT; [2]PhoenixSongs Biologicals, Branford, CT

Abstract:
Human neural stem cells (NSCs) offer advantages over established cell lines and primary rodent neurons in that they are genetically stable, scalable for molecular and chemical screening and they can be reliably differentiated into excitatory and inhibitory neurons with mature functional synapses for use in cellular assays for drug efficacy and chemical safety screens.
Here we will present how we have used human neurons produced from the robust and scalable differentiation of neural stem cells in a high throughput format to screen a chemical library to identify compounds that have potential neurotoxicity. The NSCs were expanded and differentiated in defined media containing stage specific growth factors and signaling molecules and then following 28 days of differentiation and maturation were exposed to the compounds for 24-48 hours.
The results from these studies will be presented.

Disclosures: M. Roach: None. **R. Malavarca:** None. **K. Gomes:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.09/MMM35

Topic: G.04. Physiological Methods

Support: NIMH R21 MH096233

HSCI/Millipore seed grant

Title: Establishment of a single cell assay to screen individual cell types for efficacy of putative small molecule drugs for lowering Aß

Authors: *M.-C. LIAO[1], J. LOVE[2], T. YOUNG-PEARSE[1];
[1]Harvard Med. School/Brigham and Women'S Hosp., Boston, MA; [2]Dept. of Chem. Engin., MIT, Boston, MA

Abstract: Progressive accumulation of amyloid beta (Aß) in senile plaques in the brain is a hallmark of Alzheimer's disease (AD). Aß is generated from sequential processing of amyloid precursor protein (APP) by ß-secretase followed by γ-secretase. Aß species of varying amino acid lengths are generated due to imprecise cleavage by γ-secretase within the transmembrance

domain of APP. In familial AD, mutations in APP or Presenillin1 lead to a general increase in Aß production, or more commonly to an increase in the ratio of Aß42 to Aß40. Several classes of γ-secretase inhibitors have been developed that target Aß production. Because γ-secretase cleaves a multitude of substrates in addition to APP, it is important to identify γ-secretase modulators having selective effects on the generation of Aß, but only minimal effects on other γ-secretase substrates. While Aß is generated throughout the body, neuronal cells generate high levels relative to other cell types. Very little is known about which neuronal subtypes secrete the most toxic forms of Aß. Therefore, high throughput examination of which cell types generate the highest levels of Aß, and the response of these cells to γ -secretase inhibitors would be valuable. In order to investigate Aß production from single neuronal cells, we adopted the method of microengraving, which was originally developed in the immunology field by Dr. Love's lab at MIT. First, we optimized the capture and detection antibody pairs that are specific for total Aß, Aß40 or Aß42. These antibody pairs allowed us to detect Aß production from single human APP overexpressed cell over the course of 2-4 hours in culture. Second, through comparison of the Aß level from single cells before and after treatment with different γ-secretase inhibitors (DAPT, L685,458, 31C and compound E), we observed that there are different profiles of responsiveness from single cells with different inhibitors. In addition, we evaluated Aß40 and Aß42 levels from single cells before and after treatment of different γ-secretase modulators including R-flurbiprofen and sulindac sulfate. Treatment with these modulators showed that the correlation slope of Aß42 to Aß40 was reduced after treatment suggesting that these γ-secretase modulators lowered the production of Aß42 to a greater degree than Aß40 when examined at the single cell level. Ongoing studies are examining total Aß detected from individual neuronal cells derived from human induced pluripotent stem cells. From the single cell analysis platform, we could evaluate what subtypes of neurons secrete the highest levels of Aß, and further evaluate the response of each single cell to different γ-secretase inhibitors and modulators.

Disclosures: M. Liao: None. **J. Love:** None. **T. Young-Pearse:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.10/MMM36

Topic: G.04. Physiological Methods

Title: Characterization of human fetal hippocampus-derived neural stem/progenitor cells and its application to drug discovery

Authors: *K. FUKUSHIMA, Y. IMAIZUMI, Y. TABATA, N. KOHMURA, K. YAMAZAKI, M. SUGAWARA, K. SAWADA, M. ITO;
Eisai Co., Ltd., Tsukuba-Shi / Ibaraki, Japan

Abstract: To improve the success rate of drug development, it is important to predict clinical efficacy and toxicity of candidate compounds in its upper stream with high probability. Human cells are a promising tool to be used for this purpose, but it has been difficult to utilize human cells in the neuroscience field, because of limited accessibility to human primary neurons. However, recent progress in human stem cell availability has provided solutions for this problem, such as using human iPS cells-derived and human neural stem cells-derived neurons. In this study, we characterized HIP009 cells (PhoenixSongs Biologicals, Inc.) which are human fetal hippocampus-derived neural stem/progenitor cells. HIP009 cells can be differentiated into neural cells by the cultivation in differentiation medium for ≥ 4 weeks. After the differentiation, population analysis by immunocytochemistry showed that about fifty percent of differentiated HIP009 cells were neurons and the remaining were astrocytes. mRNA expression analysis by RT-qPCR revealed that MAP2 expression was upregulated in the differentiated HIP009 cells, indicating differentiation into neurons. The analysis also demonstrated upregulation of *GRIN1* encoding NMDA receptor subunit 1, and *GRIA2* encoding AMPA receptor subunit 2. To confirm these glutamate receptor function, Ca^{2+} mobilization assay of differentiated HIP009 cells was conducted using a Ca^{2+} sensitive fluorescent dye. Fluorescent signals were increased by NMDA treatment, and NMDA-induced signals were decreased by MK-801 in a concentration-dependent manner. These results indicated that differentiated HIP009 cells expressed functional NMDA receptors. In addition, function of AMPA receptors was confirmed by AMPA and NBQX. AMPA also increased frequency of spontaneous action potential in electrophysiological recordings. These data suggest that differentiated HIP009 cells are a unique tool derived from human neural stem/progenitor cells to assess effects of compounds on human NMDA and AMPA receptors. Moreover, differentiated HIP009 cells can be more applicable to the drug screening than human primary neurons, because undifferentiated HIP009 cells can be proliferative.

Disclosures: K. Fukushima: None. **Y. Imaizumi:** None. **Y. Tabata:** None. **N. Kohmura:** None. **K. Yamazaki:** None. **M. Sugawara:** None. **K. Sawada:** None. **M. Ito:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.11/MMM37

Topic: G.04. Physiological Methods

Title: Fast-scan cyclic voltammetric measurements of tonic and phasic dopaminergic neurotransmission

Authors: C. W. ATCHERLEY[1], K. M. WOOD[2], E. B. MONROE[1], N. D. LAUDE[1], K. L. PARENT[1], P. HASHEMI[2], *M. L. HEIEN[1];
[1]Chem. and Biochem., Univ. of Arizona, Tucson, AZ; [2]Wayne State Univ., Detroit, MI

Abstract: Fast-scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes (CFMs) is a well-established technique used to correlate phasic (rapid) chemical changes to electrical, pharmacological, and behavioral stimulations. A large, non-Faradaic background current generated from high scan rates necessitates background subtraction, which makes FSCV unable to measure tonic (slow) changes. This limitation means that flow injection analysis (FIA), where a sample bolus is introduced onto the CFM, is needed for in vitro calibrations. In this work, we show that calibration using FIA underestimates neurotransmitter concentration and overestimates their duration. We describe the development of a novel method, delayed-timing voltammetry (DTV), which exploits adsorption phenomena at CFMs. We use DTV to report absolute dopamine concentrations in vivo. With FSCV, electrically stimulated, phasic dopamine changes were determined to be 560 + 60 (± SEM, n = 15 animals) and with DTV, the tonic levels of dopamine in vivo were measured as 210 ± 25 nM (± SEM, n = 15 animals). These measurements were validated with pharmacological agents. Furthermore, DTV was to develop a kinetic calibration for in vivo measurements. Because the response of carbon-fiber microelectrodes is on the same temporal order as biological events, pharmacokinetic studies have been difficult. By using a kinetic calibration the electrode response can be deconvolved from the analytical signal, thereby improving the fidelity of fast-scan cyclic voltammetry. The kinetic calibration was used to study the kinetic effects of GBR 12909, a DAT inhibitor, in mice. Whereas with traditional analysis post-GBR changes were found to be insignificant we found, using the method, it was found that the drug significantly increased Km from 0.2 to 1.0, We thereby present two novel applications for DTV; obtaining absolute neurotransmitter concentrations and for creating a more 'true' calibration profile for FSCV data.

Disclosures: C.W. Atcherley: None. **K.M. Wood:** None. **E.B. Monroe:** None. **N.D. Laude:** None. **K.L. Parent:** None. **P. Hashemi:** None. **M.L. Heien:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.12/MMM38

Topic: G.04. Physiological Methods

Support: RO1 NS055312-S1

Title: Biomechanical issues in autonomous positioning of microelectrodes in brain tissue

Authors: *S. ANAND, S. SAMPATH KUMAR, J. MUTHUSWAMY;
Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

Abstract: Advances in implantable MEMS technology has made possible adaptive microscale robotic implants that can track and record from single neurons in the brain. An adaptive micro-robotic system can enhance quality and reliability of neural recordings by automated tracking of single neurons in brain tissue in chronic experiments.
The research presented here is motivated by the need to (a) precisely position microelectrodes close to a single neuron(s) of interest in the brain so that signal to noise ratio (SNR) of the neural signal recorded is maximized and (b) to maintain the relative distance between the neuron and the microelectrode to achieve stability in the recorded signal. However, due to the viscoelastic properties of the brain tissue that has a complex non-linear time dependent response to a step displacement, moving the microelectrode by a known distance does not always directly translate to an equivalent displacement in brain tissue. The challenges to achieve precise positioning of microelectrode near a neuron include - i) no direct visual feedback of the position of the electrode ii) absence of reliable constituitve model that can fully model the inhomogeniety and variability in the mechanical properties of brain tissue at the micro-scale.
A force-feedback strategy is used in this study where the microelectrode is mounted on a force sensor and data is recorded while positioning the microelectrode in brain tissue. A quasi-steady state is defined where the forces on the microelectrode from the brain tissue reach a constant value. Constant force values indicate a steady-state relative displacement between neuron and microelectrode. Two microelectrode movement parameters were varied - i) step size of movement (3 μm - 120 μm) and ii) inter-movement interval (IMI) (30 s - 20 min). Unidirectional movement of microelectrode with the above step sizes and IMIs did not yield steady state force values after the microelectrode was positioned at the desired location. A stress relaxation model for viscoelastic materials was fit to this data. Careful analysis of the different step-sizes and IMIs showed that an inchworm type movement methodology with a forward movement of 60 μm immediately followed by a backward movement of 30 μm within a span of 1 min resulted in quasi steady-state forces. Results of testing the above optimal microelectrode movement strategy in an open-loop micro-robotic control system in short and long-term rodent experiments will be presented. The autonomous positioning system includes a ‘search’ mode for seeking neural recordings of interest and a ‘maintain’ mode for maintaining stable units once desired target neural recording has been achieved.

Disclosures: S. Anand: None. **S. Sampath Kumar:** None. **J. Muthuswamy:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.13/MMM39

Topic: G.04. Physiological Methods

Support: Royal Society Industry Fellowship to SRS

European Union FP7 289146

BBSRC BB/K001817/1

Title: Two-photon targeted robotic patch-clamp electrophysiological recording *In vivo*

Authors: L. A. ANNECCHINO, A. MORRIS, O. AGABI, P. CHADDERTON, *S. R. SCHULTZ;
Dept of Bioengineering, Imperial Col., London, United Kingdom

Abstract: Understanding the functional principles of the mammalian cortical circuit is one of the major projects of modern neuroscience. To make progress on this problem, we need to be able to observe the behavior of the individual neuronal elements of this circuit. The whole cell patch clamp technique has been the gold standard method for this, as it allows both subthreshold and suprathreshold signals to be recorded, perturbations to be applied through current injection, and delivery of DNA vectors directly into single cells. However, it has largely been limited to investigating a single neuron at a time in vivo. Considerable advantages could be gained by (i) automation and (ii) visual targeting. Recently, Kodandaramaiah et al (Nature Methods 9:585-7, 2012) developed an automated approach for performing blind, in vivo patch clamping. However, this method does not allow individual elements of the cortical circuit to be addressed. By targeting the technique to specifically labeled individual cells or cell classes (Margrie et al, Neuron 39:911-8, 2003), it may be possible to test a wide range of hypotheses concerning information processing operations performed by the cortical circuit. To make this possible, we have developed a strategy for two-photon targeting of an automated whole cell patch clamping algorithm. Our robotic patch clamp implementation was motivated by the system described by Kodandaramaiah, but has a number of key differences, including Labview implementation of a finite state machine software architecture, a closed loop pressure regulation system, and a more accurate suction procedure. The pressure regulator is controlled by the computer through a routine implementing a PID controller. In order to perform the suction task a parametric algorithm was implemented which dynamically changes the pressure set point of the PID controller, allowing greater reliability in the seal formation and break-in processes. Since pipette pull-off following single neuron targeting plays a critical role in success of transfection

procedures, our improved procedure offers the prospect of more accurate and reproducible pull-off dynamics, and thus higher hit rates in comparison to manual approaches. Our patch clamping robot is coupled to a two-photon microscope, which is used to drive neuronal target acquisition via a point-and-click graphical user interface. 3D stacks of labeled tissue are acquired, targets are selected, optical coordinates converted into the micromanipulator coordinate system, and then a suitable path calculated to feed to the patching system. Our system naturally scales to the selection of multiple identified neurons.

Disclosures: L.A. Annecchino: None. **A. Morris:** None. **P. Chadderton:** None. **S.R. Schultz:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Scientifica Ltd. **O. Agabi:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.14/MMM40

Topic: G.04. Physiological Methods

Support: KAKENHI 24223004

KAKENHI 24243067

KAKENHI 24120504

KAKENHI 248027

Title: Optimization of injection protocol for *In vivo* blind single-neuron electroporation for labeling

Authors: *K. OYAMA[1,2], Y. TATEYAMA[2], S. OHARA[2], S. SATO[2], F. KARUBE[3], F. FUJIYAMA[3], Y. ISOMURA[4], H. MUSHIAKE[1], T. IIJIMA[2], K.-I. TSUTSUI[2];
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Abstract: How individual neurons are linked to each other to form circuitries within the nervous system and how their activity leads to complex functions, such as sensory and motor processes as well as learning and cognition, are fundamental questions in neuroscience. In order to make a direct link between the morphological and functional study of the nervous system, it is necessary to label the electrophysiologically identified neuron during behavior. Previously, we have

succeeded in labeling individual neurons persistently without microscopic guidance by injecting a plasmid encoding fluorescent protein electroporatively after recording their activity extracellularly. The purpose of this study was to optimize the injection protocol for in vivo blind single-neuron electroporation for labeling. Using a glass pipette filled with electrolyte solution containing a plasmid encoding green fluorescent protein (GFP), single-neuron recording and electroporation were performed on anesthetized rats. When performing the electroporation at the completion of recording, the degree of contact between the target neuron and the electrode tip was adjusted by monitoring the change of the trace of recorded action potentials and the increase of electrode resistance. We tested two parameters of voltage pulse train (50 pulses at 50 Hz or 200 pulses at 200 Hz) and five degrees of contact (increase rate of electrode resistance from the normal baseline level; 0, 15, 30, 60, or 120%). The optimum condition for labeling was a 30% increase of the electrode resistance with voltage pulse train of 50 pulses at 50 Hz, and the labeling success rate evaluated 3 days after labeling was 40%. The rate evaluated one month after labeling using this optimum condition was only slightly lower (33%). We also confirmed experimentally that this recording and labeling procedure can be similarly successful in head-fixed behaving rats. This new experimental protocol will be a breakthrough in systems neuroscience because it makes a direct link between the morphology and behavior-related activity of single neurons.

Disclosures: K. Oyama: None. **H. Mushiake:** None. **Y. Tateyama:** None. **S. Ohara:** None. **S. Sato:** None. **F. Karube:** None. **F. Fujiyama:** None. **T. Iijima:** None. **K. Tsutsui:** None. **Y. Isomura:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.15/MMM41

Topic: G.04. Physiological Methods

Support: NIH grant R00N5051401

Title: Sensing exocytosis using an electrochemical glutamate sensor at the calyx of Held

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Abstract: A key component of neuronal communication is the ability to release neurotransmitter from presynaptic terminals. This process is mediated by the exocytosis of neurotransmitter filled vesicles from the presynaptic terminal. Current analysis of the exocytotic events from neurons in

brain slices are often made by postsynaptic electrical recordings. Fast cyclic voltammetry, or patch-capacitance at presynaptic terminals, and/or fluorescence based techniques are also frequently used. However, these techniques have some limitations. For instance, voltammetry is limited to detection of dopamine and serotonin release, while presynaptic capacitance recordings can only measure exocytosis in a limited number of large presynaptic terminals. Fluorescence techniques can have difficulties due to the need for transgenic animals, or due to low signal-to-noise ratio, and in slices they can lack spatiotemporal resolution to best analyze exocytosis. In this work, we describe an alternative approach employing a tip electrode that allows electrochemical based measurements to probe for changes in glutamate levels at physiologically relevant concentrations. Our initial experiments detecting various known glutamate concentrations in solution are encouraging for detection at concentrations from a few to hundreds of micromolar up to millimolar levels. We are now in the process of minimizing the size of the probe's tip while maintaining sensitivity for glutamate detection in an effort to optimize both parameters which is important for detection of glutamate at synapses. We are also testing the temporal sensitivity of this sensor, and its ability to detect multiple brief applications of neurotransmitter by using a fast perfusion system. In addition, we are testing other neurotransmitters, such as GABA and glycine, to determine the probes ability to selectively detect glutamate. In parallel, we are determining the ability of this probe to measure release of glutamate from calyx of Held synapses. This large presynaptic terminal allows an ideal system to investigate the ability of this electrochemical sensor to measure glutamate release from neurons in brain slices. This type of sensor would be a valuable and sensitive tool for studying glutamate release and can provide new insights into exocytotic events at excitatory glutamatergic synapses.

Disclosures: A. Kisner: None. **S. Clarke:** None. **K.G. Paradiso:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.16/MMM42

Topic: G.04. Physiological Methods

Title: A novel sealant for FSCV electrodes that is cheap, fast, easy, and reliable

Authors: *E. RAMSSON;
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Abstract: Fast-scan cyclic voltammetry (FSCV) is a powerful technique for measuring sub-second changes in neurotransmitter levels. One of the greatest limiting factors in the use of

FSCV is the production of high quality recording electrodes; the most common recording electrode consists of cylindrical carbon-fiber encased in borosilicate glass. When the borosilicate is heated and pulled, the molten glass ideally forms a tight seal around the carbon-fiber cylinder. It is often difficult, however, to guarantee a perfect seal between the glass and carbon. Indeed, much of the time spent creating electrodes is in an effort to find a good seal. To that end, many labs will utilize epoxy resins to generate a seal between the surrounding glass and carbon. While this can be effective, it is irreversible (seals cannot be adjusted), wasteful (it cannot be reused once hardener is added), hazardous (hardeners are toxic), and requires extensive curing times and/or conditions. Herein I describe the use of paraffin as a novel electrode sealant for FSCV borosilicate cylinder electrodes. Paraffin boasts the advantages of immediate curing times, resealing capability, and lack of toxicity. It is reusable, cheap, simple, and provides stable waterproof seals capable of withstanding normal mammalian body temperatures. Electrode tips were left as-is or broken and resealed with paraffin embedding wax, store-bought household paraffin wax, or epoxy resin. Excess wax was removed from the carbon surface by repeated cycling at an extended waveform (-0.4 to 1.4V, 400 V/s, 60 Hz) until the electrode size stabilized, at which point it was switched to a more standard waveform (-0.4 to 1.3V, 400 V/s, 10 Hz) and cycled until stable. Excess epoxy was removed with xylene prior to hardening and electrodes cycled at the 1.3V waveform until stable. Paraffin-sealed electrodes were just as effective at detecting dopamine as glass or epoxy-sealed electrodes, were stable, and caused a dramatic increase in the throughput of electrode production. From this it is clear that paraffin wax is an effective sealant for FSCV electrodes that not only decreases electrode production times, but provides a convenient substitute to epoxy sealants.

Disclosure.DisclosureBlock:

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585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.17/MMM43

Topic: G.04. Physiological Methods

Support: PACA region entreprise

ANR

Title: *In vivo* use of transistor for local electrical recording and glucose sensing

Authors: *T. DOUBLET[1,2,3], E. ISMAILOVA[2], L. WELCH[4], P. P. QUILICHINI[1], A. GHESTEM[1], T. HERVE[3], C. K. OBER[4], G. G. MALLIARAS[2], C. BERNARD[1];
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Abstract: Neuronal firing and field oscillations are major readouts of brain function *in vivo*. Numerous devices have been designed to record single units and field potentials, on the surface of the brain (e.g. grids) or *in situ* (e.g.
silicon probes), including in human for diagnosis purposes. These electrodes have enabled major scientific and clinical advances. However, a technological jump is now needed to allow micro scale multimodal recordings.
We now present such devices.
We developed new microelectrodes which record neuronal activities in vivo and which can be functionalize for glucose sensing. Those electrodes use OECT (organic electrochemical transistor) as recording site. Those electrodes show a more sensitive and powerful glucose sensing than the catalytic electrode. We present two models of glucose sensors based an OECT including the first use of PGMA nanobrushes attached to organic materials here demonstrated allowing a local covalent docking of the glucose oxidase without altering the conductivity of the transistor.
These new probes may constitute the basis for future local multimodal in vivo recording.

Disclosures: T. Doublet: None. **E. Ismailova:** None. **L. welch:** None. **P.P. Quilichini:** None. **A. Ghestem:** None. **T. Herve:** None. **C.K. Ober:** None. **G.G. Malliaras:** None. **C. Bernard:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.18/MMM44

Topic: G.04. Physiological Methods

Title: Experimental procedure for electrophysiological investigation of nervous conduction in murine spinal cord *In vivo*

Authors: *P. DIBAJ[1], H. STEFFENS[2], K.-A. NAVE[3], E. D. SCHOMBURG[4];
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Max-Planck-Institute for biophysical chemistry, Göttingen, Germany; [3]Neurogenetics, [4]Max-Planck-Institute for Exptl. Med., Göttingen, Germany

Abstract: Excitability and conduction of peripheral and central nerve fibers in mice gained increasing interest, since transgenic mice have been generated with alterations of neuronal and glial properties, partly resembling the clinic of human diseases. After we had investigated the conduction velocity of different nerve fiber groups in different nerves of the PNS in vivo (Steffens et al. Physiol Res 2012; 61:203-14), we now investigated characteristics of nervous conduction of peripheral and central nerve fibers from the ischiadic nerve over the dorsal root L4 to fasciculus gracilis of the spinal dorsal column. The Experiments were performed in fully anesthetized mice (initially pentobarbital i. p., continuance with methohexital i. v.). A tracheotomy was performed for artificial ventilation and electrocardiogram was recorded throughout the experiment. After paralyzation (pancuronium i. p.), changes of the heart rate and core body temperature were used to control the anesthetic state.
In the first type of experiments, we stimulated the sciatic nerve and recorded the incoming volley and dorsal column potentials at dorsal root L4 and fasciculus gracilis, respectively. Hereby, we were able to investigate simultaneously peripheral and central nerve fibers. In a second approach, in which central fibers were only investigated, stimulation was carried out at dorsal root L4 and recording was performed 7 to 10 mm cranially at the dorsal column.
In both type of experiment, the recorded compound action potentials (CAP) showed interindividually different amplitudes but comparable conduction velocities. During a period of high frequency tetanic stimulation a reliable decay of the amplitude of CAP was observed. With the stimulus frequency of 100 Hz decay of the CAP amplitude of up to 40% was observed within 10 min of tetanic stimulation. This experimental approach is of particular interest for the functional characterization of nerve fibers, not only for those with structural abnormalities but also for those with metabolic deficits due to alterations of axon-glia interaction without structural abnormalities.

Disclosures: P. Dibaj: None. **H. Steffens:** None. **K. Nave:** None. **E.D. Schomburg:** None.

Poster

585. **New Tools for Studying Channels, Receptors and Single Neurons**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 585.19/MMM45

Topic: G.04. Physiological Methods

Title: Open-ended MEMS probe for single unit recording

Authors: *S. OH, J. CHO;
Hwarangno 14-gil 5, Seongbuk-gu, KIST, Seoul, Korea, Republic of

Abstract: Behavioral single unit recording is the most advanced method for identifying neuronal functions in relation with a specific behavior. Especially, a tetrode, a bundle of four electrodes that are positioned in the form of square array, has been known to be the most efficient tool for measuring signals from individual neurons. The stereotrode or tetrode has been prepared by mechanically twisting two or four micro-sized electrodes. In addition, proper heat or adhesives should be applied to stick electrodes together. Disadvantage of this conventional method is the variation of shape and size of electrode tips due to insulation damage during heating and the extent of twisting, which can possibly reduce the efficiency of signal separation. The present study attempts to provide a most standardized way to fabricate uniform stereotrode or tetrode in terms of tip size and gap size between each electrode, which could enhance the capability of signal separation. We have applied a variety of MEMS (Micro Electro Mechanical Systems) processes including LASER technique to fabricate stereotrode. Moreover, polymers have also been used for insulation, which provides excellent biocompatibility and electrical stability. We were able to design the uniform open-ended streotrode or tetrode that can be obtained by combining two stereotrodes. Confirmations are made on the shape of open-ended MEMS probes under SEM (Scanning Electron Microscope) image and also on electrical properties of the probe.

Disclosures: S. Oh: None. **J. Cho:** None.

586**Poster**

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.01/MMM46

Topic: G.04. Physiological Methods

Support: EuroBIOSAS ICS Initative

Nano-Tera.ch Initiative

3D NeuroN project in the European Union's Seventh Framework Programme

Title: Pattern and guide - getting control over a developing neuron network

Authors: *H. DERMUTZ, R. GRÜTER, A. TRUONG, L. DEMKO, T. ZAMBELLI, J. VÖRÖS;
IBT, ETH Zurich, Zürich, Switzerland

Abstract: Neuron cultures in neuroscience are a powerful and essential tool when it comes to basic questions about network behavior and learning. In vivo, the tremendous complexity of the brain topology results in too many variable parameters influencing the basic network of interest. Many groups have started to use chemically patterned surfaces to create defined networks. These small networks are a useful tool for investigating the basic functionalities and parameters of neuron networks. We developed a method to control the location and the time of neurite outgrowth by locally switching the surface from repulsive to adhesive.
We use an AFM-cantilever with a microchannel to locally dispense an adhesion promoter onto a polymer coated glass substrate. The Poly-L-Lysine grafted Polyethyleneglycol (PLL-g-PEG, SuSoS, Switzerland) monolayer is known to be non-fouling and prevents cells from adhering to the substrate. The non-adhesive PLL-g-PEG is locally exchanged with adhesion promoting Poly-L-Lysine (PLL) when later the PLL is dispensed onto the PLL-g-PEG monolayer with the FluidFM (Cytosurge, Switzerland). After depositing PLL spots into the non-fouling PLL-g-PEG background, primary hippocampal neurons from E17 Wistar rats are dispensed over the whole substrate. The non-adherent cells are later rinsed off resulting in patterned cell spots.
Furthermore it is possible to write adhesive cues into the non-adhesive substrate with micrometer precision. This results in neurite outgrowth only in the defined direction. Control over polarity of the patterned network can be achieved with correct timing of writing the connection path between two cell spots. We have shown that the patterned network has spontaneous activity after 15 days in culture.
The system presented has flexible control over the topology of a neuron network. The possibility to change the surface from non-adhesive to attractive even when the cells are already present, makes it a very powerful tool for investigating network development and network plasticity. In addition, we have started to adapt the system to write on a standard micro-electrode array (MEA, Multichannel Systems, Germany). This will allow to overcome the limited time and spatial resolution of the calcium indicators which are used right now to investigate the network activity.

Disclosures: H. Dermutz: None. **R. Grüter:** None. **A. Truong:** None. **T. Zambelli:** None. **J. Vörös:** None. **L. Demko:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.02/NNN1

Topic: G.04. Physiological Methods

Support: NWO TOPTalent 62001113

Title: Neurodevelopment and synaptic communication in topographically connected small neuronal networks

Authors: *M. B. MARTENS[1], V. CHOKKALINGAM[2], N. NADIF KASRI[3], D. SCHUBERT[4], W. T. S. HUCK[2], P. H. E. TIESINGA[1];
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Abstract: Several neurological disorders, such as autism and intellectual disability (ID), are linked to genetic mutations that result in changes in neuronal morphology and synaptic strength. Whereas on single neuron level genetically induced synaptic deficits have been intensively investigated, it is not known how these changes can affect neural network formation and maturation. We developed a system in which small neural networks of dissociated cortical neurons of embryonic rats are compartmentalized. The compartments connected by topographically guided axon projections through polydimethylsiloxaan (PDMS) microtunnels. By culturing the neurons on planar multi electrode arrays (MEAs), we tracked the formation of the topographically connected neural networks over time by (a) recording the spatiotemporal aspects of their spontaneous action potential firing, which took the form of network bursts and silent periods. (b) Recording the stimulus evoked synaptic signal propagation to a neural network in a neighboring compartment. In our setting, electrical activation of one neural network resulted in propagation of activity to the connected neural networks. Synaptic recruitment of network activity is delayed by 50 millisecond for a compartment directly connected to the stimulated compartment. Synaptic recruitment for networks connected through a relay neural compartment, a delay of 100 millisecond is recorded. The compartments are electrically stimulated as well as optically stimulated by using the light-gated channelrhodopsin ionchannels. We also tracked the network maturation and the role of single ID-linked genes by recording the same neural networks from day-in-vitro (DIV) 7 up to DIV 32 and show how the spontaneous network bursts develop as the network matures.

Disclosures: M.B. Martens: None. **V. Chokkalingam:** None. **N. Nadif Kasri:** None. **D. Schubert:** None. **W.T.S. Huck:** None. **P.H.E. Tiesinga:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.03/NNN2

Topic: D.05. Visual Sensory-motor Processing

Title: *In vivo* 3D measurement of neuronal network activity during visual stimulation

Authors: *K. SPITZER[1], G. SZALAY[2], G. KATONA[2], P. MAÁK[3], M. VERESS[3], A. KASZÁS[2], L. SULCZ-JUDÁK[2], B. CHIOVINI[2], D. PÁLFI[4], B. RÓZSA[2];
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Abstract: In order to study the memory process and neuronal network activity in a less modified environment *in vivo* experiments are required. Although several electrophysiological methodologies and imaging techniques have been developed for 3 dimension network measurements none of them provided the high spatial and temporal resolution that can be realized with 3D two-photon acousto optical imaging.
We used a high-resolution, acousto-optic two-photon random-access scanning microscope that reaches near-cubic-millimeter scan range (up to 700 × 700 × 1,400 μm^3), with a high scanning speed, with 470 × 490 × 2,490 nm^3 resolution in the center core, and less than 1.9 x 1.9 x 7.9 μm^3 resolution throughout the whole scanning volume, which is still sufficient to measure activity from the cell somatas in the whole scanning volume. With these tools we measured the neuronal activity of hundred of cells in the visual cortex in 3D with millisecond temporal resolution.
We show volumetric random-access scanning calcium imaging of visual stimulation-evoked activity in hundreds of neurons of the mouse visual cortex in *in vivo* head restrained mice.

Disclosures: K. Spitzer: None. **G. Szalay:** None. **G. Katona:** None. **P. Maák:** None. **M. Veress:** None. **A. Kaszás:** None. **L. Sulcz-Judák:** None. **B. Chiovini:** None. **D. Pálfi:** None. **B. Rózsa:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.04/NNN3

Topic: G.04. Physiological Methods

Support: NIH Grant R01NS050452

NIH Grant R01EB009429

Title: Development of a functional assay for investigating human neural network plasticity *In vitro* toward the integration with a “body-on-a-chip” device

Authors: *B. J. BERRY, M. T. SCHNEPPER, N. AKANDA, X. GUO, J. J. HICKMAN; Nanoscience Technol. Ctr., Orlando, FL

Abstract: The pre-clinical research and drug development process of bench to bedside to public is an expensive and slow process. Furthermore, the translation of drug effects from animal trials to clinical research is often poor, with clinical trials failing in humans after promising results were obtained in animal models. This gap in the translation of results in animal studies to human clinical trials could be closed with the use of robust human-based in vitro test beds. To this end, we are working to construct a human-on-a-chip in vitro system which models human tissue from multiple organs for measuring drug effects. One component of this large-scale system will model the central nervous system's in vivo structure by recapitulating layered neural networks. Previous work has characterized the initial establishment of this system, and validated the methodology employed to achieve a functional in vitro analogue of the layered neural networks observed in the in vivo central nervous system using rat cells (Natarajan et al. 2013). Human stem cell-derived pyramidal neurons are now being investigated for their ability to reach functional maturity, as measured using standard electrophysiological techniques. Cells were capable of producing substantial inward Na+ currents and outward rectifying K+ currents following 21 days in vitro when maintained in a defined, serum-free environment on a controlled, self-assembled monolayer (SAM) surface. Chemical patterning of cytophilic and cytophobic SAMs facilitated the establishment of patterned surfaces for the control of cell attachment and neuritic outgrowth. Using this technology, surfaces were patterned to produce neural populations arranged into layers feeding forward to subsequent layers, thereby mimicking the cellular architecture of the in vivo tissue. Microelectrode arrays were used to monitor these layered networks for evidence of network connectivity over time. Such cultures were stimulated using defined electrical protocols in order to induce changes in synaptic strength and thereby produce in vitro analogues of phenomena such as long-term potentiation (LTP). Dynamic changes in synaptic strength are continuously occurring in vivo with neurodegeneration occurring when this process breaks down. Inhibition of LTP is an indicator of serious neurodegenerative diseases, such as Alzheimer's. This study focuses on the development of a system whereby LTP can be induced, maintained and altered, with and without drug treatment, as an indicator of network health and viability. With the development of an in vitro system which can predict clinical drug effects, we hope to improve future medical research and drug development.

Disclosures: B.J. Berry: None. **M.T. Schnepper:** None. **X. Guo:** None. **J.J. Hickman:** None. **N. Akanda:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.05/NNN4

Topic: G.04. Physiological Methods

Support: NIH Grant 5R21MH093858-02

Title: Oxygen polarography and electrophysiology in the default-mode and dorsal-attention networks during rest and stimulation: Bridging BOLD fMRI and electrophysiology

Authors: *W. J. BENTLEY[1], J. LI[1], A. SNYDER[2], M. RAICHLE[2], L. SNYDER[1];
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Abstract: Blood oxygen level dependent (BOLD) fMRI is our primary window into the functioning human brain. Unfortunately, the neural activity underlying BOLD remains unclear. We developed a microelectrode-based oxygen polarographic system capable of recording tissue oxygen simultaneously at 4 sites in awake macaques with 30-100 micron spatial specificity and 20 Hz sampling. Tissue oxygen is in equilibrium with blood oxygen. Thus this system can be considered a high resolution alternative to BOLD (Thompson 2003, Lowry 2010). This tool can be implemented in awake monkeys alongside standard electrophysiology and provides an unprecedented platform for high resolution study of BOLD and the neural-BOLD link. We recorded neural activity and oxygen from posterior cingulate (PC) and area V3 in awake macaques during both resting state and 15 s of 1 Hz wide-field textured stroboscopic illumination. These regions, in the default and dorsal attention networks, respectively, behave very differently. In PC, a transient increase in oxygen at the start of visual stimulation was followed by a sustained drop to a level below baseline. In V3, a large initial oxygen transient was followed by a sustained level above baseline, ending with a second smaller transient at the cessation of stimulation. In the resting state both areas show oxygen fluctuations with a 1/f power spectrum, similar to resting state BOLD. These fluctuations were correlated among all recording sites, but more strongly between bilateral within-network pairs (r=.6) than between out-of-network pairs (r=.4). Correlation strength was frequency dependent (peak at .05 Hz) suggesting that correlation involves a subset of the mechanisms driving the local fluctuations. In PC, local field potentials (LFP) and multi-unit activity show a very brief (compared to oxygen) initial peak followed by a sustained suppression. In V3, LFP and MUA track each stroboscopic flash. The response to the first flash is greater than that to subsequent flashes, consistent with the oxygen response, but there is no obvious response to the cessation of stimulation. The magnitude of the LFP response is on average 3-4 times greater in V3. In contrast, the absolute value of the oxygen response is similar in both regions (~±2%). Analysis of the average transfer function from LFP to oxygen shows different latencies, magnitudes, and waveforms in the two regions as well as different LFP frequency contributions. Together these observations argue for substantially different neural-bold links in these two regions.

Disclosures: W.J. Bentley: None. **J. Li:** None. **A. Snyder:** None. **M. Raichle:** None. **L. Snyder:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.06/NNN5

Topic: G.04. Physiological Methods

Support: NIH Grant R01-DC009215

Title: Precise control of neural network structural connectivity in neural cultures

Authors: *J. R. GAMBLE[1], J. A. MAURER[2], D. L. BARBOUR[1];
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Abstract: Neuronal interactions occur in the context of non-random structural connectivity and distributed network activity, but these dynamic interactions are challenging to study in vivo due to the overall complexity of the system. Although neuronal cultures have been utilized as a reduced system for the probing of neuronal function, the random structures inherent in these traditional cultures limit the investigation of network structure/function relationships. For example, cultured random neural networks can be trained without specific synaptic constraints to perform experimenter-defined tasks; conversely, specific synapses can be potentiated through spike-timing dependent plasticity (STDP). In order to elucidate the neural network architecture bridging these two phenomena, experimenters must be able to reliably and precisely control the structure of the cultured networks. Photolithography and microcontact printing (μCP) have been used recently in conjunction with surface chemistry to pattern neural networks of arbitrary geometries in vitro. More specifically, these techniques have been used to create low-density functional neuronal circuits, but the directionality of the axons within the networks could not be controlled. Single isolated neurons have been patterned with controlled directionality for axon differentiation by stamping self-assembling alkanethiols onto gold. However, the small feature sizes necessary to develop high-fidelity control are difficult to reproduce with traditional microstamping techniques due to problems associated with high feature aspect ratios. We have extended this latter work in combination with other μCP techniques such as submerged μCP and composite polydimethylsiloxane (PDMS)/h-PDMS stamps, which have both been shown to increase reproducibility of high aspect ratio features. This modification increases pattern feature fidelity at the single micron level and enables creation of neural networks of predetermined structural connectivity. Our current results indicate that the precise shapes of the individual

neuronal patterns are critical for neuronal compliance to the desired configuration. The starburst shaped pattern previously used for axon differentiation encourages the correct placement of somas compared to a unipolar pattern, in which somas tend to adhere to regions intended for axons. The success of this project will enable the ability to culture neural circuits of predefined geometry and directionality, providing precise control of the context of connectivity during the study of cell-cell interactions such as synaptic plasticity.

Disclosures: J.R. Gamble: None. **J.A. Maurer:** None. **D.L. Barbour:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.07/NNN6

Topic: G.04. Physiological Methods

Support: Funded by Newcastle University

Title: High frequency blocking of central pathways in the primate motor system

Authors: *K. M. FISHER[1], N. JILLANI[2], S. N. BAKER[1];
[1]Inst. of Neurosci., Newcastle Univ., Newcastle-upon-Tyne, United Kingdom; [2]Inst. of Primate Res., Nairobi, Kenya

Abstract: Transient blocking of nerves using a high frequency electrical stimulus has previously been demonstrated in the peripheral nervous system. Such reversible block of peripheral nerves has enormous clinical potential for blocking pain, muscle spasticity and unwanted bladder sphincter activity in spinal cord injury patients. Successful blocking of central pathways could have similar potential, both in the experimental field (to induce specific, transient and reversible lesions) and also in the attempt to block the unwanted signals associated with some neurological diseases.
Here, we demonstrate the first successful application of a high frequency blocking stimulus to the central nervous system using a sharp metal electrode. Experiments were performed in 3 healthy adult male olive baboons (*Papio Anubis*; 22.5-25.8kg) maintained under terminal general anaesthesia.
A laminectomy was performed to expose spinal segments T1-C5 and the underlying dura was removed to allow access to the spinal cord. Stainless steel macroelectrodes were fixed in a caudal section of the exposed spinal cord (~C6 level, dorsolateral funiculus) targeted to produce a maximal antidromic response in motor cortex via corticospinal pathways. A stainless steel movable electrode was then inserted into a more rostral region of the spinal cord (~C5 level) to

serve as the blocking electrode. This was mounted in a micromanipulator to enable slow advancement into the cord to test the blocking stimulus at multiple depths.
Stimulation was delivered through the caudal electrode as the movable 'blocking' electrode was advanced into the spinal cord. At each depth, high frequency sinusoidal blocking stimuli were delivered at a range of intensities (200-1000µA) and frequencies (2-10kHz). An epidural recording over M1 was observed throughout to determine the proportion of the antidromic field which was blocked.
Results in all 3 animals showed a frequency and intensity specific effect of blocking. High intensity, low frequency (2kHz) sinusoidal stimuli were most effective at blocking the antidromic field and were capable of near-complete blockade at the optimal depth.
In conclusion, we have demonstrated transient, reversible blocking of central motor pathways using a high frequency sinusoidal stimulus. This technique could be a valuable tool for experimenters wishing to induce reversible lesions in central nervous pathways.

Disclosures: K.M. Fisher: None. **S.N. Baker:** None. **N. Jillani:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.08/NNN7

Topic: G.04. Physiological Methods

Support: ECS-12081804

ECS-1002391

Title: An improved M-Sorter for automatic and robust spike sorting

Authors: *S. WANG, Y. YUAN, W. MA, J. SI;
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Abstract: Neural spike detection and classification, or spike sorting, is the first and a critical step prior to any single unit based neuroscientific studies and applications. A good spike sorter is usually characterized by high detection and classification accuracy, robustness to changes in signal-to-noise (SNR) ratio, objectivity in results or less user dependency, and real-time applicability. The M-Sorter is an automatic and robust spike detection and classification system, based on the multiple correlation of wavelet coefficients (MCWC) detection algorithm in conjunction with k-Means and template matching for classification. Extensive tests have been performed using the M-Sorter and compared with several popular algorithms, the thresholding

detection and T-distribution EM classification of the Offline Sorter by Plexon and the open source code of Wave Clus. Note that the sorters we compared were under the automatic modes since our aim was to perform automatic spike sorting. Therefore we did not compare manual spike sorting with Offline Sorter or any other sorter. The sorters under consideration were comparable under high SNRs conditions while the M-Sorter showed advantage in low SNRs. Also, the M-Sorter results appeared more consistent and less sensitive to parameter changes.
Two improvements were made to the improved version of the M-Sorter for enhanced user experience. First, time efficiency is greatly improved by utilizing parallel computation toolbox in the Matlab. This toolbox is available for computers equipped with multi-core processors. Tests were made using Dell Optiplex 7010 with Intel Core i7-3770 and 16GB ram. As a result, running time is reduced to 50% of the previous version. For example, for a single channel dataset of 1 hour long, it takes 97 seconds to sort with resulted firing rate of 71 spikes/s.
Second, a user-friendly interface is developed and implemented. This allows the user to view the clustering results from the automatic mode of the M-Sorter, merge clusters, and save results. In addition, the principal components, averaged waveforms, inter-spike intervals (ISIs) are also available as guidance to the user when choosing clusters.
This M-Sorter is available online for free download.

Disclosures: S. Wang: None. **Y. Yuan:** None. **W. Ma:** None. **J. Si:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.09/NNN8

Topic: G.04. Physiological Methods

Support: Max Planck Society

Title: Coupling between spiking activity and beta band spatio-temporal patterns in the macaque PFC

Authors: S. SAFAVI[1], *F. PANAGIOTAROPOULOS[2], V. KAPOOR[1], N. LOGOTHETIS[1], M. BESSERVE[1];
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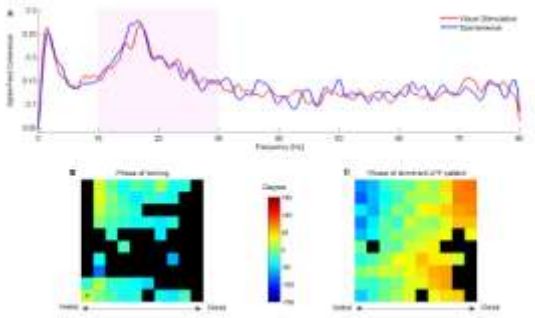
Abstract: Previous analysis of Local Field Potentials (LFPs) recorded from the inferior convexity of the macaque prefrontal cortex (PFC) revealed a dominant travelling wave pattern in the beta band (15-30 Hz) propagating along the ventral-dorsal plane. We hypothesized that

propagating rhythmic activity reflects the intrinsic dynamics of the underlying neural populations which might be instrumental to information processing and sensory integration. Here, we investigated the relationship between multi-unit spiking activities (MUA) and LFPs in the same area of the PFC.
We first computed spike-field coherence for each channel of the array. Many recording sites (typical example in Fig 1A) exhibited a distinctive peak in the beta frequency range both for resting state (spontaneous activity) and during visual stimulation with dynamic movie stimuli. We extracted the instantaneous phases in the beta band using Hilbert transform. We then computed the phase locking of spikes in each channel to a common LFP reference channel. The results exemplified on Fig 1 B, showed that many recording sites exhibited locking of spikes to the same phase of remote beta band LFP. This result was observed for many LFP reference channels, suggesting action potentials in all channels are synchronized to a common phenomenon.
We used complex Singular Value Decomposition (SVD) of the spike-phase locking matrix to capture the dominant underlying spatio-temporal pattern of beta oscillations associated to spiking activity across the array. The dominant pattern estimated from the first eigen-mode of SVD exhibits a phase gradient along the ventral-dorsal plane (Fig 1 C), suggesting that MUA across the array are synchronized to the global travelling wave pattern previously observed along this direction in the LFP signal.
This new result suggests MUA is synchronized to large scale ongoing travelling wave patterns in the beta band both during stimulation and spontaneous activity. Further information theoretic analysis will address how this mechanism serves distributed sensory encoding and processing in this area.



Disclosures: S. Safavi: None. **F. Panagiotaropoulos:** None. **M. Besserve:** None. **N. Logothetis:** None. **V. Kapoor:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.10/NNN9

Topic: G.04. Physiological Methods

Support: FONDECYT proyecto regular 1110369

BFU2010-19149

This work was supported by the Millenium Center for the Neuroscience of Memory, Chile, NC10-001-F, with Innovation for Competitivity funds from the Ministry for Economics, Fomentation and Tourism, Chile

Title: Online detection of ripple events and theta waves in the hippocampus *In vivo*

Authors: *N. GRAVEL[1,1], J. HURTADO[1], P. FUENTEALBA[1,2];
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Abstract: Fast online detection of LFP activity patterns has become an essential tool to understand the neural correlates of a variety of cognitive processes. In the hippocampus, high frequency (100-300 Hz) sharp wave ripples, an activity pattern that has been associated with memory formation and recall, can be disrupted by early pattern detection followed by feedback stimulation. Being highly localized in time and frequency, early ripple detection pushes the limits of reliability and sensitivity. In contrast, lower frequency signals, such as theta activity (5-9 Hz), are less localized in time, allowing for a long observation period before a more reliable and accurate detection can be made. In both cases, most detection methods used so far depend on parameters that are tuned heuristically by the investigator, after inspecting a large sample of the time series. However, the parameter space for automatic detection systems is large, and an exhaustive search is hard to achieve. We present online and offline detection methods especially devised for ripple events and theta oscillatory activity. We used signal detection theory to quantitatively compare the performance of online versus offline detection algorithm using human experts as "gold standard". Receiver operating characteristics and precision-recall curves were constructed to evaluate the performance of the detectors and a systematic search of parameter space was undertaken to find regions of optimal performance. Our results reveal that false hits pose a major methodological issue in many online detection algorithms and that a quantitative approach to parameter selection can largely improve the design of precise, high recall detection systems.

Disclosures: N. Gravel: None. **J. Hurtado:** None. **P. Fuentealba:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.11/NNN10

Topic: G.04. Physiological Methods

Support: NSF Grant IOS 0622318

NIH Grant EY003039 (CORE)

Oak Ridge Associated Universities (ORAU)

Title: The effect of temporal stimulus waveform on the cortical steady-state visual evoked potential (SSVEP)

Authors: *T. J. GAWNE[1], T. DICKERHOFF[1], W. J. KRAFT[2], K. Q. CHANG[1];
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Abstract: The cortical steady-state visual evoked potential (SSVEP) is simply flickering or flashing a visual stimulus and recording the electrical response from visual cortex. The SSVEP has been used for decades, but it is still an active area of research. However, there is little data on the effects of temporal stimulus waveform on the SSVEP. Researchers typically just use whatever stimulus is available: brief flashes for strobe lights, single brief pulses or trains of brief pulses for CRT displays, and LCD displays can exhibit a variety of temporal waveforms. In particular, with conventional paradigms it is difficult to change stimulus waveform or frequency without also changing mean luminance. Here we constructed an LED-based visual stimulator that, by using pulse-width modulation at 5 kHz, could generate visual displays with different frequencies and temporal waveforms but the same mean luminance. The display was a diffuse white disk divided into four quadrants, and subtending 20 degrees in visual angle. Subjects fixated the center of the disk. The conditions were two seconds each of null control, square wave at 10 and 20 Hz, and 10% duty cycle pulse at 10 and 20 Hz. The conditions were presented in random shuffled order with a variable inter-trial interval. Signals were recorded from a novel three-electrode array centered over the occiput. Preliminary results from four subjects indicate that the effects of changing stimulus waveform while keeping the mean luminance constant is idiosyncratic between subjects and frequencies. Sometimes there was no difference in the SSVEP using square wave or pulsatile stimuli, and sometimes the differences were marked. Nonetheless, there was a trend for the pulsatile stimulus waveform to generate stronger phase-locked responses especially at higher frequencies. Using another protocol where mean-luminance matched pulses of 1,2,4, and 8 msec durations were presented at 15 Hz indicates that small changes in the shape of a pulsatile stimulus have negligible effects on the SSVEP, except for a small increase in response phase with increasing pulse width. These results suggest that it may be difficult to compare the results of different SSVEP studies that use high- or low- duty

cycle stimulation, but that small differences in the waveforms of a pulsatile visual stimulus likely have little effect.

Disclosures: T.J. Gawne: None. **T. Dickerhoff:** None. **W.J. Kraft:** None. **K.Q. Chang:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.12/NNN11

Topic: G.04. Physiological Methods

Title: Optimizing EEG/EMG signal/noise ratio

Authors: R. KOZMA[1], *W. J. FREEMAN, III[2], C. T. LIN[3], L.-D. LIAO[3];
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Abstract: Aim: Our goal is to extract information on higher cognitive functions from the noninvasive scalp electroencephalogram (EEG).
Introduction: The human scalp EEG contains massive information that is correlated with higher cognitive functions. Samples taken from arrays of electrodes show that the information is in the form of spatiotemporal patterns of briefly stationary bursts of electric potential differences (1). The bursts are generated by masses of cortical neurons located 10-30 mm below the scalp surface. They are signals that are contaminated by electrical noise from scalp muscles located 2-5 mm beneath the scalp surface.
Methods: We oversample the mix of EEG signals and electromyographic (EMG) noise, calculate temporal and spatial power spectral densities (PSDt and PSDx), calculate temporal and spatial Nyquist sampling frequencies, and write temporal and spatial filters in software to optimize the temporal and spatial pass bands to extract neural correlates of cognition (1).
Results: We designed and constructed an electrode array to oversample EEG+EMG by 48 closely spaced electrodes in a flexible curvilinear array that was quickly fixed on the scalp in any orientation (2). The spacing was based on preliminary analyses of the spatial frequencies imposed on the scalp EEG by the gyri and sulci of the cortex (3); the typical width and length of gyri are on the order of 10 and 30 cm, giving a spatial Nyquist frequency of 0.2 cycles/mm. The temporal Nyquist frequency of 2000 Hz was based on the need for temporal precision in measurements of the phase of signals in the high gamma and epsilon ranges, respectively 30-80 Hz and 80-200 Hz.
Conclusions: Previous studies (4) have shown that the PSDt of the EEG is 1/fA, where the

exponent is 2<A<4 (5), while on average the EMG PSDt conforms to 1/fB, where B = 0. Therefore the EMG imposes a plateau onto the combined PSDt, with an inflection at a high frequency, fH, on transit from 1/fA to 1/ fB above fH. Subjects can be trained by biofeedback, on seeing the PSDt, to minimize EMG and reveal the signals in the upper gamma and epsilon ranges.
A low pass spatial filter can attenuate the EMG. The cut-off spatial frequency is calculated by the spatial autocorrelation function (6), which has a spike at zero lag, which is attenuated by spatial averaging.
1 Freeman WJ, Quian Quiroga R (2013) Imaging Brain Function with EEG, Springer.
2 Liao L-D (2012) Proc IEEE 100 (13): 1553-1566
3 Ramon et al. (2009) http://escholarship.org/uc/item/9mm328md
4 Freeman et al. (2003) http://escholarship.org/uc/item/67x7q43f
5 Freeman and Zhai (2009) http://escholarship.org/uc/item/7wf8544n
6 Freeman et al. (2010) Abstr #738, Proc 16th Conf Human Brain Map, 6-10 June

Disclosures: R. Kozma: None. **W.J. Freeman:** None. **C.T. Lin:** None. **L. Liao:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.13/NNN12

Topic: G.04. Physiological Methods

Support: NIH NS45248

Title: A small-volume recirculating bath using a modified airlift pump provides both oxygenation and flow for *In vitro* electrophysiological studies

Authors: *M. L. MCKINNON, S. HOCHMAN;
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Abstract: In vitro electrophysiological studies allow for bath application of drugs at known concentrations without impediment of the blood-brain barrier. Oxygenation is provided via perfusion systems to maintain tissue viability. This typically involves tubing and external reservoirs with relatively large volumes of fluid. Many pharmacological studies require the use of expensive drugs so minimization of recirculation volume becomes important.
We have designed a small-volume self-contained recirculating perfusion chamber utilizing the action of a modified airlift pump. Physiological saline solutions are simultaneously oxygenated and pumped through the chamber at very high flow rates (60 mL/min). The device may be

quickly fabricated using acrylic sheets cut with an automated laser cutter, allowing for rapid production. Pieces are subsequently bonded with dichloromethane for assembly. The current design allows for a minimum of 8 mL of fluid, but may be scaled up to larger volumes to suit the individual application. There are no mechanical or electrical components and an internal design minimizes bath level fluctuations, thereby reducing noise.
A previous study used the airlift pump for low volume recirculation (Wilson et al 1999; J Neurosci Methods. 87:175-184). Our design additionally allows for incorporation of a simple external mechanism for rapid solution exchanges (< 1 min). Solution exchange is performed by inserting a flow bypass circuit into the bath outlet to decouple recirculation. Using this chamber we have been able to demonstrate increased activity in rat spinal cord following bath application of serotonin, and a subsequent decrease in activity following drug washout after 2 hours in vitro. We anticipate use of this chamber in studies where high flow rates and expensive pharmacology are required.

Disclosures: M.L. McKinnon: None. **S. Hochman:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.14/NNN13

Topic: G.04. Physiological Methods

Support: NIH Grant NS052233

Title: Toward a self-wired active reconstruction of the hippocampal trisynaptic loop: DG-CA3

Authors: *B. C. WHEELER[1], M. D. BOEHLER[2], S. LEONDOPULOS[1], L. PAN[1], S. ALAGAPAN[1], T. B. DEMARSE[1], G. J. BREWER[2];
[1]Biomed. Engin., Univ. of Florida, GAINESVILLE, FL; [2]Med. Microbiology, Immunol. and Cell Biol., Southern Illinois Univ. Sch. of Med., Springfield, IL

Abstract: The mammalian hippocampus functions to encode and retrieve memories by transiently changing synaptic strengths, yet encoding in individual subregions for transmission between regions remains poorly understood. Toward the goal of better understanding the coding in the trisynaptic pathway from the dentate gyrus (DG) to the CA3 and CA1, we report a novel microfabricated device that divides a micro-electrode array into two compartments of separate hippocampal network subregions connected by axons that grow through 3x10x400 um tunnels. Gene expression by qPCR demonstrated selective enrichment of separate DG, CA3 and CA1 subregions. Reconnection of DG to CA3 altered burst dynamics associated with marked

enrichment of GAD67 in DG and GFAP in CA3. Surprisingly, DG axon spike propagation was preferentially unidirectional to the CA3 region at 0.5 m/s with little reverse transmission. Therefore, select hippocampal subregions intrinsically self-wire in anatomically appropriate patterns and maintain their distinct subregion phenotype without external inputs.

Disclosures: B.C. Wheeler: None. **M.D. Boehler:** None. **S. Leondopulos:** None. **L. Pan:** None. **S. Alagapan:** None. **T.B. DeMarse:** None. **G.J. Brewer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BrainBits LLC.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.15/NNN14

Topic: G.04. Physiological Methods

Support: NSF Grant ECS-1002391

NSF Grant ECS-12081804

Title: Accurate and automatic spike classification based on robust EM algorithm

Authors: *W. MA, H. MARKLEY, J. SI;
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Abstract: Spike classification is an essential step in the analysis of single unit neural activities recorded using multi-channel electrodes. In this study we propose an accurate and automatic spike classification method named the accurate robust expectation-maximization (AREM) algorithm. It is a novel interleaving two-step approach. The AREM is initialized by the K-means. Then the following two steps are performed recursively in time. The first step is to compute the optimal clustering results once the number of clusters is given using the conventional robust EM algorithm. The other step, which is also our innovation, is to eliminate an unnecessary cluster per iteration until an optimal number of clusters is reached. This step is important for obtaining robust and improved clustering.
For eliminating unnecessary clusters, a pair of the most similar clusters is first identified, which corresponds to the largest overlapping area of two clusters that are above a certain threshold. The smaller cluster of the pair is then eliminated. The final optimal number of clusters is considered to be the one that corresponds with when the negative change in the log-likelihood performance measure is above a threshold.
The AREM and some popular sorters are compared for performance evaluation. The EM sorting

and the valley-seeking methods in Plexon's Offline sorter (Plexon Inc.) are considered, as well as the Superparamagnetic Clustering in Wave Clus (Quiroga et al., 2004). These specific algorithms are chosen for comparisons because they are all in the automatic spike sorting category.
First, two artificial spike data sets were used. All three sorters have comparable total accuracy and misclassification rates for data with high signal-to-noise (SNR) ratios and low similarities among clusters. But the AREM outperformed others on both false positive and false negative rates for difficult data sets with low SNR, high similarity among clusters, and occasional overlapping spikes.
Additionally, the AREM was used in conjunction with the M-Sorter (Yuan et al., 2012) to replace the semi-automatic K-means clustering method. Two real neural data sets recorded from rat's motor cortex were used for performance testing. Comparisons among the updated M-Sorter with AREM classifier, the Plexon's Offline sorter, and the Wave Clus reveal that the updated M-Sorter with AREM not only maintains its overall accuracy, but also has less required design parameters, resulting in a high performance automatic sorter.

Disclosures: W. Ma: None. **H. Markley:** None. **J. Si:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.16/NNN15

Topic: G.04. Physiological Methods

Support: NSF CCF-0939370

Salk Institute Innovation Award

Title: A novel method to assess event-related brain potentials (ERP) in clinical domains using frontal epidermal electronics (EES) sensors

Authors: *R. GIL-DA-COSTA[1], R. FUNG[1], S. KIM[2], D. MESA[2], R. MA[2], D. KANG[2], M. BAJEMA[2], T. D. ALBRIGHT[1], T. P. COLEMAN[2];
[1]Salk Inst. For Biol. Studies, LA JOLLA, CA; [2]Bionengineering, UCSD, San Diego, CA

Abstract: We have previously reported the development of an epidermal electronics system (EES), and its use to capture event-related brain potentials (ERP). The EES is no thicker than a human hair, flexible, stretchable, and mechanically matched to the skin. We further investigated the use of EES together with advanced signal processing techniques, to detect and measure the mismatch negativity (MMN), an ERP modulation widely correlated with neuropsychiatric

disorders. The MMN is thought to reflect pre-attentive detection of a deviant stimulus and can be calculated as the difference wave between the responses to deviants (infrequent) and to standard (frequent) stimuli in an oddball paradigm. Neuropsychiatric patients suffering from a variety of disorders, such as schizophrenia, Alzheimer's disease or autism spectrum disorder, appear less able to detect novel stimuli than healthy controls. Consistent with this behavioral deficit, the amplitude of MMN has been found to be reduced. This has led to the proposal that a reduced MMN is a marker of progressive pathology or vulnerability for these disorders. Currently, there is a two-fold limitation for a wider and successful application of these findings in neuropsychiatric research and therapy, arising from: i) the difficulty of testing using a traditional electroencephalography (EEG) cap and ii) the analytical limitations associated with lower signal-to-noise ratios when attempting to obtain successful evaluations at the individual level.
Here, we show the development of a novel minimally obtrusive forehead sensing technology, using EES, together with a novel analytical approach, a "neuroscience-guided test", for MMN detection in healthy subjects. A group of 14 subjects were tested with both: (a) a traditional EEG full cap and typical statistical analysis and (b) our novel EES frontal sensors and methodology. Our results show that while a traditional approach, selecting an optimal electrode (Cz), can detect a significant MMN ($p < 0.01$) in only 9 subjects, our novel non-obtrusive, easy to apply methodology can detect a MMN ($p < 0.01$) in 11 subjects. As such, our proposed novel method combining forehead EES sensors with a "neuroscience-guided test" brings a striking advance at both i) ease of testing and ii) increased individual detection. We believe that these methodology developments can open new avenues for clinical research and therapeutic intervention opportunities across a myriad of neuropsychiatric and neurological disorders. Future studies should assess the efficacy of this methodology on detection and quantification of modulations of the MMN, as well as other ERPs of interest, in different patient populations.

Disclosures: R. Gil-Da-Costa: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroVerse, Inc.. **S. Kim:** None. **D. Mesa:** None. **R. Ma:** None. **D. Kang:** None. **M. Bajema:** None. **T.P. Coleman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroVerse, Inc.. **R. Fung:** None. **T.D. Albright:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.17/NNN16

Topic: G.04. Physiological Methods

Support: National Digital Research Centre

Science Foundation of Ireland (J.K)

Title: The development and characterisation of new software that integrates behavioral tracking with real time oxygen monitoring using a wireless implantable telemetry system in freely moving rats

Authors: *E. M. GARRY[1], C. LLOYD[2], R. BENNETT[3], J. KEALY[4], J. LOWRY[4], R. GEOGHEGAN[3];
[1]Blue Box Sensors Limited, Dublin, Ireland; [2]ANY-maze, Stoelting Co., Chicago, IL; [3]Blue Box Sensors, Dublin, Ireland; [4]NUI Maynooth, Kildare, Ireland

Abstract: We have developed new software that allows simultaneous monitoring of data from an implanted oxygen sensor in the brain which is directly integrated into ANY-maze behavioral tracking software to create a new and powerful data collection tool.
This system involves the selective detection of dissolved oxygen (O2) in vivo by amperometry using a pre-calibrated carbon paste electrode (CPE, diameter 200 µM) that responds within O2 physiological concentrations (50-80 µM). The O2 sensor is implanted in the hippocampus and is connected to a wireless telemetry transmitter which is implanted in the abdomen. This system is fully implanted subcutaneously and can remain in the animal with continuous data collection possible for >6weeks.
This is therefore ideal for behavioral and social interaction experiments but no software that could integrate tracking and physiological data from the same source was available. Data from the implanted telemeter is received from a charging pad which allows telemeter recharging while implanted. This charging pad is connected to a data acquisition system that contains the software for acquisition of the oxygen signal. Behavioral tracking data was monitored with separate ANY-Maze software making synchronisation of both data sets difficult. We designed software that integrated the oxygen signal directly into ANY-maze behavioral tracking software to allow complete synchronisation of both signals as well as data analysis.
To validate the system, we have tracked the behaviour of implanted rats in different behavioral mazes and simultaneously monitored oxygen. We show that this integrated tracking and telemetry system is a valid way to examine real-time changes in brain tissue oxygen during spatial and information processing tasks.
This system supersedes existing telemetry systems with a fully integrated behavioral tracking and data analysis package making this a powerful tool for long term in vivo monitoring of tissue oxygen in rat brain.

Disclosures: E.M. Garry: None. **C. Lloyd:** None. **R. Bennett:** None. **J. Kealy:** None. **J. Lowry:** None. **R. Geoghegan:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.18/NNN17

Topic: G.04. Physiological Methods

Support: Belgian National Funds for Scientific Research (FNRS)

EU-grant-FP7-ICT-2011-9, n. 600806 "Corticonics"

PUR 2009 and ''Dote ricerca'': FSE, Regione Lombardia (University of Milan); Prin2008 (Italian Government)

Fonds Léon Fredericq

James S. McDonnell Foundation

Mind Science Foundation

European Commission (Mindbridge, DISCOS, DECODER & COST)

Title: Quantification of brain complexity to assess the level of consciousness

Authors: *O. GOSSERIES[1,2], A. CASALI[3], M. ROSANOVA[3], M. BOLY[4,2], S. SARASSO[3], K. CASALI[5], S. CASAROTTO[3], M.-A. BRUNO[2], S. LAUREYS[2], G. TONONI[4], M. MASSIMINI[3];
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Abstract: ***Objectives***: A fundamental shortcoming of current clinical practice is the lack of a reliable method to objectively assess the level of consciousness. Theoretical considerations suggest that consciousness depends on the ability of neural elements to engage in complex activity patterns that are, at once, distributed within a system of interacting cortical areas (integrated) and differentiated in space and time (information-rich). The aim of the study is to test a novel measure of complexity, the Perturbational Complexity Index (PCI), based on transcranial magnetic stimulation combined with electroencephalography (TMS/EEG) measurements to assess the level of consciousness in single individuals across different conditions.
Methods: We used TMS-EEG in healthy subjects during wakefulness, dreaming, NREM sleep,

different levels of sedation induced by different anesthetic agents (midazolam, xenon and propofol), as well as in severely brain-injured patients (vegetative/unresponsive, minimally conscious, and conscious states including locked-in syndrome). In total, 208 sessions were performed in 52 subjects. PCI was used to calculate the amount of information contained in the integrated response of the corticothalamic system to a direct perturbation using algorithmic complexity measures, such as the Lempel-Ziv complexity index.
Results: PCI allowed discriminating between consciousness and unconsciousness in single individuals across the different physiological, pharmacological and pathological conditions. PCI was sensitive to graded changes in the level of consciousness, and was stable across stimulation parameters such as intensity or site. Regarding the patients with brain injury, we stimulated preserved brain regions as the stimulation of brain-damaged areas induced no response.
Conclusions: This theoretically motivated quantification of brain complexity allows establishing an objective, graded measurement scale along the consciousness/unconsciousness spectrum and provides a principled approach for estimating objectively the level of consciousness at the bedside. The use of a neuronavigation system is strongly advised to assess the level of consciousness in patients recovering from coma.

Disclosures: O. Gosseries: None. **A. Casali:** None. **M. Rosanova:** None. **M. Boly:** None. **S. Sarasso:** None. **K. Casali:** None. **S. Casarotto:** None. **M. Bruno:** None. **S. Laureys:** None. **G. Tononi:** None. **M. Massimini:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.19/NNN18

Topic: G.04. Physiological Methods

Support: DFG MO930/4-1

Lichtenberg Grant 86 507

Title: Quantifying signal degradation caused by different spike removal techniques: Examples from human micro-electrode recordings

Authors: *M. BAYRAKTAR[1], V. A. COENEN[2], C. E. ELGER[1], F. MORMANN[1];
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Abstract: Single neurons interact with the neuronal network they are embedded in. Thus, the firing behavior of a single neuron can be modulated by the oscillatory activity of the Local Field Potential (LFP) of the surrounding neuronal network, a phenomenon described as phase locking. Methodological approaches to detect and quantify phase locking of a neuron's action potentials (spikes) to the raw or filtered LFP can yield false positive results due to the spectral properties of spikes extracted from the same LFP signal. Several recent studies have implemented preprocessing techniques to remove spikes before examination of spike-LFP synchronization in order to avoid false positive results induced by action potentials. In this study, we show that common techniques for removal of action potentials can have spurious effects on the detection of phase locking.

We studied the effects of four spike removal techniques: linear interpolation, mean spike removal, cubic spline interpolation and Bayesian spike removal. We applied each technique to extracellular microwire recordings obtained from epilepsy patients undergoing seizure monitoring prior to resective surgery. To test for false positive detections of phase locking we created phase-randomized surrogate signals from the original LFPs and used these in combination with the original spike trains. To scan for false negative effects, we used the surrogate LFP signals in combination with artificial spike trains designed to show a predefined amount of phase locking. Phase locking detection was implemented using phase extraction by Hilbert transformation and subsequent statistical testing by the Kuiper's test. We studied effects of spike removal on raw LFP signals as well as from different frequency ranges from the delta to the fast ripple band.

We analyzed recordings from four microwires in the human medial temporal lobe. Our results show that even for analyses in low frequency ranges, spike removal by both linear and cubic spline interpolation causes significantly high false positive and false negative effects on the phase locking detection, whereas mean spike and Bayesian spike removal have significant impact only on frequency ranges above 100 Hz.

Judging from our preliminary findings, we reason that spike removal can lead to wrong interpretation of phase-related metrics, particularly for high frequency bands such as ripple and fast ripple activity. Mean spike removal and Bayesian spike removal techniqes appear far more reliable than linear and cubic spline interpolation.

Disclosures: M. Bayraktar: None. **V.A. Coenen:** None. **C.E. Elger:** None. **F. Mormann:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.20/NNN19

Topic: G.04. Physiological Methods

Title: Eye fixation-related potentials for natural event processing in a computer interaction

Authors: *F. COURTEMANCHE, P.-M. LÉGER, S. SÉNÉCAL, A. ORTIZ DE GUINEA, R. TITAH, M. FREDETTE, É. L. LEMOYNE;
HEC Montreal, Montreal, QC, Canada

Abstract: The eye-fixation related potential (EFRP) method allows the synchronization of eye tracking with electroencephalographic (EEG) recording to precisely capture individuals' neural activity at the exact time at which they start to cognitively process a natural stimulus while interacting with a computer (e.g., event on the screen). This method is well suited to register neural activity while individuals use a computer device in a multitasking environment. This method complements and addresses some of the shortcomings of the traditional Event Related Potential (ERP) method. For example, the traditional ERP can only stamp the time at which a stimulus is presented to an individual, not the time at which the individual cognitively processes it. An experiment was conducted with twenty-four healthy university students to demonstrate that the processing of the stimulus is phase-locked to the fixation rather than its presentation. During the experiment, participants were asked to read a business report with a computer screen while email pop-up notifications arrived on their screen. The participants then had to open (or not) the emails if they thought they were relevant for their report reading task.. EEG was measured during the task with 32-electrode array geodesic sensor net using Netstation acquisition software and EGI amplifiers. A Tobii X-60 eye tracker was used to record participants' eye movement patterns during the experiment. The Noldus Observer XT was used to synchronize the EEG and eye-tracking data by sending a TTL signal to the EGI amplifier and a keystroke signal to the Tobii Studio v 3.2. The results showed three distinct neural processes associated with a) the cognitive reaction to email pop-up notification (a P300 component), b) the cognitive processing of the email pop-up notification (a N400 component identified with the EFRP method), and c) the motor planning activity involved in opening or not the email (a BP component). Furthermore, post-hoc analyses showed the criticality of the EFRP method to isolate the neural activity associated with stimulus processing by illustrating that such activity cannot be obtained with the ERP method alone. The original and distinctive characteristic of this technique is that it allows, by using eye-tracking in conjunction with EEG, to stamp the exact time at which a particular natural stimulus is processed by an individual, rather than the time at which the stimulus is presented to this individual.

Disclosures: F. Courtemanche: None. **P. Léger:** None. **S. Sénécal:** None. **A. Ortiz de Guinea:** None. **R. Titah:** None. **M. Fredette:** None. **É. L. Lemoyne:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.21/NNN20

Topic: G.04. Physiological Methods

Title: Change in functional connectivity by continuous whisker stimulation correlates with decrease in LFP power correlation in the delta band

Authors: *H. LU[1], L. WANG[2], W. REA[2], E. A. STEIN[2], Y. YANG[2];
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Abstract: Spontaneous fluctuations in the resting state fMRI time course exhibit structured spatial and temporal patterns. However, the underlying mechanisms of the fluctuations are still poorly understood. The goal of this study is to investigate how tasks modulate ongoing spontaneous fluctuations in resting state fMRI and electrophysiological signals. SD Rats (n=6) were scanned at a 9.4T scanner under two conditions: 1) continuous whisker stimulation (sinusoidal waveform; frequency: 2, 4 or 6 Hz; peak-to-peak amplitude: 4 mm); 2) resting state (no stimulation). fMRI time courses were low-pass filtered (< 0.1 Hz). Functional connectivity between bilateral whisker barrel cortex (WBC) was analyzed using cross-correlation. In comparison to the resting condition, continuous whisker stimulation decreased bilateral WBC functional connectivity only at 2Hz, but not at 4 or 6 Hz. In a separate experiment, epidural EEG recordings were performed from bilateral WBC, with visual cortex (VC) serving as a control site (n=10). Time-frequency analysis was conducted using Morlet wavelet transformation. Power time course correlations between recording sites were calculated. Whisker stimulation significantly augmented EEG power from alpha to gamma frequency range, but decreased power only in the delta band. Furthermore, for all frequencies (2, 4 or 6Hz) examined, whisker stimulation significantly decreased power correlations between bilateral WBC from beta to low gamma ranges. Notably, only with the stimulation frequency of 2 Hz did we observe a decrease in delta band power correlation between bilateral WBC. Finally, there was no significant change in any of the frequency bands between either side of WB and VC.
In summary, stimulating whiskers at 4 and 6 Hz changed bilateral WBC power correlation in high frequency LFP (beta to low gamma), but did not change WBC functional connectivity; however, stimulating whiskers at 2 Hz changed both low (<4 Hz) and high frequency LFP power correlations, and WBC functional connectivity was reduced. Our data support the hypothesis that ongoing spontaneous activity and task-evoked activity may reflect two at least partially separable processes which can be differentially registered by both the fMRI and the electrophysiological signals. More specifically, ongoing spontaneous activity seems to be registered in low frequency LFP (<4 Hz), stimulations that modulate LFP within this frequency window could modulate functional activity as revealed by fMRI; but modulating high frequency LFP signal has minimal effect on fMRI functional connectivity.

Acknowledgement: This research was supported by the Intramural Research Program of the NIH, NIDA

Disclosures: H. Lu: None. **L. Wang:** None. **W. Rea:** None. **E.A. Stein:** None. **Y. Yang:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.22/NNN21

Topic: G.04. Physiological Methods

Title: P300- like event related potentials in rat brain have a latency between 650 and 850 msec

Authors: *W. D. KLIPEC[1], J. BOWDEN[2], L. PHILLIPS[2], R. LEWIS[2], T. GRAY[2], A. PAJSER[2];
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Abstract: The human P300 event-related potential (ERP) is a time-locked response to rare, response-relevant stimuli. Alterations in amplitude, latency and lateralization have been shown in early onset Alzheimer's disease and schizophrenia. Since rat models of these diseases have been developed, a reliable rat P300-like model would be a useful tool in studying both the etiology of these conditions and efficacy of treatment models. In our laboratory, we have reported robust P300-like ERPs to task relevant stimuli in freely behaving rats, but the latency and amplitude of these ERPs has been highly variable. With closely spaced events, the ERPs for the events may merge forming a compound ERP that makes reliable identification of the peak voltage difficult. Here, within a standard operant lever-pressing paradigm for food reinforcement, we have systematically manipulated the inter-event intervals both for a tone that predicts the click of a food delivery mechanism, and a different tone that predicts the insertion of the lever. By tracking the latency shifts in the ERP to the predictive stimulus and the ERP to the predicted event, we have been able to separate what appears to be late auditory ERPs at about 240-340 msec from much larger ERPs that are P300-like but reliably occur at latencies between 650-850 msec. The amplitude of these events is consistent with our earlier research that suggested these ERPs represent correlates of conditioned reinforcement. This rat P300-like model has significant potential as a research tool for investigating both Alzheimer's and schizophrenia using a rat model.

Disclosures: W.D. Klipec: None. **J. Bowden:** None. **L. Phillips:** None. **R. Lewis:** None. **T. Gray:** None. **A. Pajser:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.23/NNN22

Topic: G.04. Physiological Methods

Support: U.S. Army Contract: W911NF-10-S-0002-0005

U.S. Army Cooperative Agreement: W911NF-12-2-0019

Title: Validation of a real-world multi-aspect integrated neuroimaging system

Authors: W. D. HAIRSTON[1], *T. J. DOTY[1], B. KELLIHAN[2], J. CANADY[2], K. W. WHITAKER[1], K. S. OIE[1], K. MCDOWELL[1];
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Abstract: The multi-aspect integrated neuroimaging system described here is a brand new, first-of-its-kind system designed to monitor changes in neurological, physiological, behavioral, and subjective experience in real-world environments. This project is the first attempt at simultaneous long-term monitoring and integration of all four aspects of human function outlined above, and includes four different devices that must work in concert to collect data in real-time. The system consists of a high density, wireless, dry electrode EEG cap to measure neurological output throughout a normal day; a bioharness worn around the chest for measuring heart rate variability and movement; a sensor worn on the wrist that measures skin conductance; and an Android-based portable electronic device (PED) carried by the participant which integrates wireless data from the other devices, displays scheduled inventories, and provides the participant with an
interaction panel to log relevant events throughout the day. By having all components synchronized with a PED, we are afforded the unique opportunity to integrate contextual information about real-world daily activities which is often lacking from previous studies. Namely, context is created 1) objectively from integrated physiological sensors (e.g. measuring cardiac and skin conductance fluctuations) and 2) subjectively from an event monitoring panel on the PED which the user interacts with at intervals throughout the data collection period. This robust collection of data comes, however, with the necessity of precise synchronization of equipment, and verification that the approach is not only comfortable but also non-intrusive for use within daily life.
Here we discuss the methods developed to validate the performance of the system and its ability

to be used within a standard office environment over the course of several complete work days. This is a critical step because, given the novelty of this approach within the neuroimaging field, no current guidelines exist for system evaluation. Examples include use of phantom devices for quantifying integration, analytical methods for evaluating system performance and stability of the components over time, and metrics for general comfort and usability, such as the Comfort Visual Analog Scale (VAS) and assessments of burden (from the Burden VAS). Taken together, we aim to establish feasibility of real-world neuroimaging through the approaches described here, which employ synchronized context monitoring while minimizing discomfort and burden on the user.

Disclosures: W.D. Hairston: None. **T.J. Doty:** None. **B. Kellihan:** None. **J. Canady:** None. **K.W. Whitaker:** None. **K.S. Oie:** None. **K. McDowell:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.24/NNN23

Topic: G.04. Physiological Methods

Support: DARPA SBIR W31P4Q-13-C-0141

Title: Portable EEG device and mobile application for mTBI assessment

Authors: *K. S. HALE, M. JOHNSTON, B. D. WINSLOW;
Design Interactive, Inc., Oviedo, FL

Abstract: Electroencephalography (EEG) is a well-established non-invasive technique for neurological monitoring with high temporal resolution and relatively low cost. As such, EEG has proven to be a critical monitoring and diagnostic tool in the clinic. EEG is also a popular research tool among psychologists and neuroscientists for evaluating somatosensory responses to stimuli, error detection, and sleep monitoring, among other uses. An emerging role for EEG is a first-line measure or triage tool for brain injury or stroke in situations where access to high spatial resolution imaging technologies is not readily available. In the immediate post-injury phase several abnormal electrophysiological signatures are apparent in the EEG, including decreased delta and theta amplitudes, low coherence, and reduced asymmetry in eyes-closed recordings. Current approaches to treat sports or blast-related injuries consist of care center-based imaging and neurosurgical procedures when necessary. However, there are no devices available to support field triage of affected individuals and assess severity immediately following injury. Here we present miniaturized, wireless, high-performance EEG, which consists of the

integration of recording and transmission hardware within a small, lightweight form factor, with low power consumption. The device streams data to a mobile device, which allows for immediate, non-expert data visualization and interpretation. To aid users without significant EEG experience, software applications were developed to support placement, data interpretation and visualization. The montage application also features augmented reality sensor placement and an anatomical landmarking support tool to aid users without significant EEG experience. Following 5 minutes of eyes-closed recordings, the software detects the presence or absence and type of mTBI.

Disclosures: K.S. Hale: None. **M. Johnston:** None. **B.D. Winslow:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.25/NNN24

Topic: G.04. Physiological Methods

Support: Kentucky Spinal Cord and Head Injury Research Trust

Title: Enhancements to a non-invasive system for high-throughput monitoring of mouse sleep and other behaviors

Authors: M. STRIZ[1], M. SETHI[1], T. ZHANG[2], H. L. CANTER[1], J. BRIGHAM[1], S. JOSHI[1], R. GOOCH[3], M. GOPALAIAHGARI[1], F. YAGHOUBI[4], K. D. DONOHUE[3], S. SUNDERAM[4], *B. F. O'HARA[2];
[1]Biol., [3]Electrical and Computer Engin., [4]Biomed. Engin., [2]Univ. of Kentucky, Lexington, KY

Abstract: Traditional methods of characterizing sleep and wake in rodents suffer from substantial limitations. EEG/EMG is invasive and time consuming, while wheel-running and other activity monitors do not distinguish sleep from low activity periods. We developed a noninvasive, high-throughput piezoelectric system that distinguishes sleep and wake in mice with high accuracy. A piezoelectric pad placed at the bottom of the mouse cage records gross body movements, while a computer classifier analyzes features of the signal and differentiates sleep from wake. Sleep signals are characterized by regular 3 Hz rhythms, as well as several other features, while wake signals exhibit variable amplitude and frequency. The classifier correlates 90-95% with EEG and human observation. This system has been used in a variety of studies, including genetic studies of sleep-related traits, and characterizing sleep in mouse models of traumatic brain injury, Alzheimer's disease and Parkinson's disease.
We are expanding the system to identify other mouse behaviors. We performed human

observation, as well as EEG and piezoelectric recordings, of C57BL/6J mice, the most common inbred strain used in biomedical research, and CFW mice, a common outbred strain. High correlation between all three methods in identifying sleep serves as a control to validate the effectiveness of human observation in identifying other behaviors. We then use human observation as a basis for identifying quiet wake, grooming, rearing, feeding, drinking, and locomotion. When these behaviors are identified, we isolate the piezoelectric signals from those time points and train the classifier to find features that reliably distinguish each behavioral domain.
Distinguishing these behaviors through the piezoelectric system would improve our phenotyping and behavioral assessments in a wide variety of projects where our system is being used_including the analysis of mouse models of traumatic brain injury, Alzheimer's Disease, Parkinson's Disease, and genetic studies designed to find genes and alleles that influence these behavioral traits.

Disclosures: **M. Striz:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **B.F. O'Hara:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **M. Sethi:** None. **T. Zhang:** None. **H.L. Canter:** None. **J. Brigham:** None. **S. Joshi:** None. **R. Gooch:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **M. Gopalaiahgari:** None. **F. Yaghoubi:** None. **K.D. Donohue:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **S. Sunderam:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.26/NNN25

Topic: G.04. Physiological Methods

Support: NIH Grant NS16541

NIH Grant NS077348

Title: Validation of automated pruritus sensing in mice and rats

Authors: ***J. C. SKAHEN**, M. MARINO, S. MALKMUS, Y. SHTAERMAN, T. L. YAKSH; Anesthesia Res. Lab., UCSD Anesthesia Res. Lab., La Jolla, CA

Abstract: Pruritus, the sensation that leads to the desire to or act of scratching, is present in a diverse number of clinical settings. Currently, pruritus is addressed by a limited number of treatments and further elucidation of its mechanisms will have great clinical significance. This

work characterizes and validates a paw motion detector (PMD) automated system for mice and rats. By detection of the movement of a removable metal band affixed to the hind paw inside an electromagnetic (EM) field, the PMD allows for the rapid automated analysis of animal motion. An important issue relates to the validation of the automated scratch detection algorithm with the counting determined by the trained observer. Male C57 Bl6 mice and male Holtzman Sprague Dawley rats were fitted with a small metal hind paw band and adapted individually to an EM producing and detecting chamber. In mice and rats, ID (intradermal) injection of 48/80 in the dorsolateral aspect of the upper shoulder induced high frequency scratching at the injection site with the ipsilateral (banded) hind paw. This motion was recorded via the PMD system and video camera for analysis. Animal motion was analyzed for scratch counts using various machine detection algorithms that varied over the following parameters: Signal Smoothing, Amplitude, and Frequency of motion. Based on this process, algorithms which proved optimal for accurate signal detection from the machine were noted. In mice, the scratch counts after ID saline and 48-80 were 39 ± 3.59 and 258.8± 26.7, respectively (mean ± SEM). In rats the counts after ID saline and 48-80 were 55.7± 13.45 and 298.6 ± 77.61, respectively (mean ± SEM). Linear regression between the optimal algorithm for rat and mouse scratching and trained human observation were then produced. Following ID injection of 48/80, slopes of the regression relating human observer versus machine counts using the optimal algorithm were 1.022± 0.024 in the mouse and 1.009 ± 0.069 in the rat (mean ± SEM). In additional studies various pharmacology was characterized showing that 48/80 response was blocked by diphenhydramine whereas chloroquine was not. These results show the PMD permits an accurate automated assessment of pruritus.

Disclosures: J.C. Skahen: None. **M. Marino:** None. **S. Malkmus:** None. **Y. Shtaerman:** None. **T.L. Yaksh:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.27/NNN26

Topic: G.04. Physiological Methods

Support: Inoue Enryo Memorial Foundation for Promoting Sciences of Toyo University

Title: Development of an evaluation method that analyzed the motor performance among elderly people using handwriting features

Authors: *T. ISHIZAKI[1], K. TOKUTAKE[2], T. WATANABE[2], S. ARIOKA[2], E. TANAKA[2], T. ANME[2], H. KAWAGUCHI[3];

[1]TOYO UNIVERSITY, Oura-Gun Gunma, Japan; [2]Univ. of Tsukuba, Ibaraki, Japan; [3]Toyo Univ., Gunnma, Japan

Abstract: Elderly people show a gradual decrease in motor performance caused by the progressive loss of skeletal muscle strength and mass. Control of motor performance is important for the maintenance of physical and social health, namely, quality of life (QOL), in elderly people. Therefore, we aimed to establish an easy quantitative method to evaluate the motor performance of elderly people by analyzing their handwriting features through the use of a "digital pen." This device, which digitizes handwriting at a rate of 13 ms and has a spatial resolution of 0.3 mm, can be advantageously used in our evaluation method because it can acquire data from many users easily and simultaneously. In addition, we used surface electromyography (sEMG) to measure muscle activity. sEMG of the arm was performed similar to a blood pressure manometer. A total of approximately 300 elderly people from the Joso Project (Elderly People Health Empowerment Project in Joso City), which was performed in cooperation with Joso City, University of Tsukuba and Toyo University, participated in our study. The participants were required to use digital pens to complete a questionnaire on lifestyle, memory recall and drawing a wood. We analyzed several handwriting features, including writing speed and pressure. The handwriting data were compared with their physical and motor performance. As a preliminary study, four participants (two men, 55 and 83 years of age; two women, 55 and 87 years of age) completed the questionnaire using digital pens. We measured skeletal muscle mass and handgrip strength and conducted the chair-stand test in four participants. sEMG data obtained from each participant's dominant arm were based on the weight of water-filled PET bottles (0-2 L). We determined the correlations between handgrip strength and average writing speed, as well as the correlations between the chair-stand test results, average writing speed, and stroke rate. sEMG data revealed that the muscle activity of healthy individuals differed from that of individuals with sarcopenia syndrome. Thereafter, we will continue to analyze the data acquired from the Joso Project.
The protocols used in this study were approved by the Ethics Committee of University of Tsukuba. This study was partially supported by Inoue Enryo Memorial Foundation for Promoting Sciences of Toyo University.

Disclosures: T. Ishizaki: None. **K. Tokutake:** None. **T. Watanabe:** None. **S. Arioka:** None. **E. Tanaka:** None. **T. Anme:** None. **H. Kawaguchi:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.28/NNN27

Topic: G.04. Physiological Methods

Title: Assessment of central sensitization in chronic pain patients: The influence of crosstalk on reflex receptive field mapping

Authors: *M. B. JENSEN, J. BIURRUN MANRESA, O. K. ANDERSEN;
Aalborg Univ., Aalborg Ø, Denmark

Abstract: Recent studies indicate that evaluation of the human withdrawal reflex is useful for assessing central sensitization. Enlarged reflex receptive fields (RRF) have been detected in groups of chronic pain patients compared to control groups, most likely reflecting expansion of spinal neuronal receptive fields. The RRF of muscles in the lower extremities can be quantified by randomised, distributed electrical stimulation, typically on the sole of the foot. Each muscle or group of synergistic muscles has a unique cutaneous RRF where noxious stimulation may elicit a reflex in that specific muscle. Interestingly, the RRFs of the two antagonistic muscles tibialis anterior (TA) and soleus (SOL) exhibit substantial overlap, in contrast to what is predicted by the modular organization theory. Most likely, this observation reflects limited specificity of the single differential (SD) EMG recordings used to quantify the reflexes, meaning that crosstalk from relatively strong reflexes in TA dominates the EMG recorded over SOL.
To evaluate this assertion, this study mapped RRFs for both TA and SOL using three different approaches to detect reflexes; evaluation of SD EMG, evaluation of double differential (DD) EMG and a novel conduction velocity analysis (CVA) specifically developed to reject crosstalk during reflex detection (Jensen et al. 2013). Reflexes were elicited by five sweeps of noxious electrical stimulation of 10 sites on the sole of the foot of 17 chronic pain patients lying in supine position, a position rendering genuine reflex activity in SOL unlikely. The area of the RRF was quantified as the fraction of the foot where a reflex was elicited by at least 25% of the stimulations. Results are expressed as median (quartiles).
In 15 of the 17 subjects, reflexes were identified from the SD EMG recorded over SOL. Moreover, the RRF mappings for SOL based on evaluation of SD EMG resemble to a great extent the corresponding mappings for TA. The RRF area for SOL was 0.17(0.00,0.51) when derived from SD EMG, whereas DD EMG (0.00(0.00,0.06)) and CVA (0.00(0.00,0.00)) entailed significantly smaller and nearly non-existing RRF areas. A markedly smaller but significant reduction in RRF area was observed for TA when reflex detection was based on CVA, from 0.34(0.17,0.53) for SD EMG and 0.37(0.20,0.53) for DD EMG to 0.28(0.17,0.45) for CVA.
These results support the notion that crosstalk may significantly influence mappings of RRFs based on evaluation of SD EMG. However, this issue can readily be reduced by the use of DD EMG or CVA, both of which constitute valuable measures to deal with crosstalk and enable improved mapping of RRFs for specific muscles.
Jensen et al. BMC Neuroscience 2013, 14:39

Disclosures: M.B. Jensen: None. **J. Biurrun Manresa:** None. **O.K. Andersen:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.29/NNN28

Topic: G.04. Physiological Methods

Support: JSPS KAKENHI (grant no. 24650431)

Title: Predicting mental health disorders based on the time intervals between strokes while writing numbers

Authors: *H. KAWAGUCHI, S. TAKISE;
Toyo Univ., Gunma, Japan

Abstract: The number of patients with mental health disorders has recently increased. Because the recurrence of mental health disorders in such patients is high, there is a social need for a method that predicts the onset of mental health disorders. Therefore, we investigated whether the Uchida-Kraepelin test, one of the tests to examine the character in stressful situations, could provide a method for predicting mental health disorders using the handwriting features of participants. We analyzed the participants' handwriting using a "digital pen," which can digitize handwriting at a rate of 13 ms and has a spatial resolution of 0.3 mm. During this test, participants were asked to perform two sets of single-digit additions for 15 min each (with a 5-min rest period between each set). A total of 201 students (who were initially aged 18-19 years) were recruited for a follow-up cohort study conducted over 3 years (once per year, in early April) and who voluntarily underwent the Uchida-Kraepelin test and answered a mental health questionnaire, General Health Questionnaire, 30 items (GHQ30). We analyzed the time intervals between the first and second stroke of a number (4, 5, and 7; mean time interval: t_1) and the time intervals between finishing the writing of a number and starting the writing of the next number (mean time interval: t_2). The ratio of the mean time interval (t_2/t_1) for people with mental health disorders was significantly higher than that of healthy individuals. In addition, a correlation was observed between the t_2/t_1 indicator and the anxiety and tension scores on GHQ30 ($p < 0.001$ for the first year, $p < 0.05$ for the second year). Furthermore, the dropout rate of the high-risk group (the group with $t_2/t_1 > 11$) was much higher than that of low-risk group (the group with $t_2/t_1 \leqq 11$; $p < 0.01$). These results suggest that it may be possible to predict mental health disorders using the t_2/t_1 indicator.
The protocols used in this study were approved by the Ethics Committee of Toyo University.
This study was partially supported by JSPS KAKENHI (grant no. 24650431).

Disclosures: **H. Kawaguchi:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; JSPS KAKENHI (grant no. 24650431). **S. Takise:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.30/NNN29

Topic: G.04. Physiological Methods

Support: American Heart Foundation Postdoctoral Fellowship

NIH Grant R21NS081438

NIH Grant R01AA018316

Title: A novel test of motor and other neurological dysfunctions in mice

Authors: ***A. M. BARTH**, I. MODY;
Neurol., UCLA Sch. of Med., Los Angeles, CA

Abstract: Like most human neurological disorders, corresponding mouse models present multiple deficiencies. Therefore, estimating disease progression or potential treatment effectiveness in such models necessitates the use of multiple time-consuming tests that usually require a large number of scarcely available genetically modified animals. To overcome this shortcoming, we wanted to develop a single, multipurpose test for functional evaluation of motor and other neurological deficits in mice.
Our approach extracts complex, 3-dimensional data from video recordings by a single camera. We investigated 3 groups of mice with various neurological deficits: 1) mice with unilateral stroke in the forelimb motor cortex; 2) mice injected with a moderate dose of ethanol (1.6 g/kg, i.p.); and 3) old (96-99 weeks of age) mice.
We show that post stroke recovery can be divided into separate stages based on strikingly different characteristics of motor function deficits deriving from compensatory mechanisms. We distinguished motor impairments with early-onset transient as well as gradually developing and persistent characteristics. We also identified motor impairments after stroke, which resemble the human motor neglect syndrome. Mice treated with a moderate dose of ethanol and old mice also showed characteristic motor and exploratory deficits.
Our novel experimental approach provides qualitatively sophisticated and complex information

about motor impairments and locomotor/exploratory activity. It should be useful for the detailed characterization of a broad range of human neurological disease models in mice, the more accurate assessment of disease progression, and treatment effectiveness.

Disclosures: A.M. Barth: None. **I. Mody:** None.

Poster

586. **New Tools for Studying Neural Networks**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 586.31/NNN30

Topic: G.04. Physiological Methods

Title: Biocompatibility analysis by histological techniques of nanostructured materials in the central nervous system

Authors: *P. R. ARTEAGA-LOPEZ[1], A. MORA LAZARINI[3], I. SANCHEZ-JERONIMO[2]; [1]Biol. of Reproduction, [2]Biol. Sci. and Hlth., Univ. Autonoma Metropolitana Iztapalapa, Mexico city, Mexico; [3]Nanomaterials, CINVESTAV, Mexico city, Mexico

Abstract: The innovation in the area of pharmacology are focusing the attention at novel innovative delivery systems and treatments based on nanotechnology devices that will improve the chemotherapeutics results and diminish the secondary effects. With the aim to improve this nanotechnology we evaluated the biocompatibility of the new devices in brain. We used rats as a model of study.
Adult intact male intact rats were used, then microinjected with nanostructured materials, as titanium dioxide bilaterally in the brain by stereotaxic method. There was no mortality in any rat during the treatment. Then the rat was perfused with PFA 4% and the brain extracted in cold conditions and stored in glucose/thymerosal solution 1% at 4oC for one week. The brain slices were obtained using a cryostat at -17oC, with a grossor thick of 8 um, then the brain were mounted in a microscope slide coated with poli-l-lisine (0.1%) and dehydrated with an alcohol series, followed by a specific stain process (nissl, hematoxiline-eosine and trypan blue). The microphotographies were taken with a light microscope (Olympus IX81) accopled with a digital camera. The biocompatibility analysis shown that the material microinjected in the brain, has not negative effects over the tissue and cell integrity because the histological analysis demonstrates that the membrane and nucleus are normal, the neurons and the membrane are intact. Those results confirm those reported previously in our laboratory. In conclusion the nanostructured titaniium dioxide used as a vector for the drug delivery is biocompatible with brain tissue and it suggest that this kind of nanomaterials are secure because it has no effect over the system.

Disclosures: P.R. Arteaga-Lopez: None. **A. Mora Lazarini:** None. **I. Sanchez-Jeronimo:** None.

587**Poster**

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.01/NNN31

Topic: G.06. Computation, Modeling, and Simulation

Support: CNRS

INSERM

College de France

ANR (ComplexV1)

European Community (BrainScales, Magnetrodes)

Title: Microscale impedance measurements are consistent with diffusive electrical properties of extracellular media

Authors: C. BEDARD[1], J.-M. GOMES[1], Y. ZERLAUT[1], M. NELSON[2], S. VALTCHEVA[3], L. VENANCE[3], P. POUGET[2], T. BAL[1], *A. DESTEXHE[1];
[1]CNRS, Gif-sur-Yvette, France; [2]ICM, Paris, France; [3]Col. de France, Paris, France

Abstract: The electric properties of the extracellular medium are still subject to a controversy because of contradictory measurements. One possibility is that the use of metal electrodes as current sources in previous studies provides non-physiological results. We tested this possibility by performing impedance measurements in conditions as close as possible to physiological conditions. We generated single-cell local field potentials (LFP) by injecting subthreshold inputs in single neurons using patch-clamp recordings, combined with extracellular recordings with micropipettes. Various measurement configurations, such as current-source or voltage-source, provided contradictory results, either low-pass or high-pass filters. Although these results never showed resistive extracellular properties, their apparent contradictory character could prevent any conclusion about the exact nature of the extracellular and intracellular impedances. To clarify this, we developed a theoretical model based on Maxwell equations, which shows that all measurements can be explained if the extracellular medium is of diffusive type (Warburg impedance). This model predicts that the phase difference between intracellular and extracellular signals should provide a signature of the physical nature of the impedance, with 45 degrees phase

difference for diffusive type. Preliminary experiments show that indeed, the phase is centered around 45 degrees, therefore confirming the diffusive nature of the extracellular impedance. In a companion poster, we provide evidence for diffusive media from EEG and MEG measurements (Dehghani et al. - this conference). These findings have potentially important consequences for interpreting LFP measurements and source estimation such as CSD analysis.

Disclosures: C. Bedard: None. **A. Destexhe:** None. **Y. Zerlaut:** None. **J. Gomes:** None. **T. Bal:** None. **M. Nelson:** None. **P. Pouget:** None. **S. Valtcheva:** None. **L. Venance:** None.

Poster

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.02/NNN32

Topic: G.06. Computation, Modeling, and Simulation

Support: Utah Governor's Office - TCIP

Title: Computational model of a charge steering DBS electrode

Authors: *A. WILLSIE, A. D. DORVAL;
Biomed. Engin., Univ. of Utah, Salt Lake City, UT

Abstract: Deep Brain Stimulation (DBS) is used to alleviate some of the symptoms associated with neurodegenerative disorders. Targets for DBS are located deep in the brain, are typically small, making targeting difficult, and have unique 3D geometries. Clinical electrodes used for DBS are limited by their geometry, cylindrical with four ring contacts, to produce roughly spherical areas of activation. Targeting and target shape can limit the ability of the clinical electrode to deliver adequate stimulation while controlling the effects of charge spread outside targets, which may drive side effects. We introduce a computational model of a new electrode geometry with thousands of contacts, capable of charge steering. Modeling shows that the new electrode can deliver stimulation to the unique geometries of deep brain targets more completely than the clinical electrode. Additionally, displacements from optimal target placement, which directly impact the utility of the clinical device, are mediated by the charge steering ability of the new electrode geometry.

Disclosures: A. Willsie: None. **A.D. Dorval:** None.

Poster

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.03/NNN33

Topic: G.06. Computation, Modeling, and Simulation

Support: IP2011 030971

N N303 542839

FP7-PEOPLE-2010-ITN 264872 NAMASEN

POIG.02.03.00-00-018/08 POWIEW

POIG.02.03.00-00-003/09 BIOCENTRUM OCHOTA

Title: The effects of realistic conductivity distribution and slice geometry on simulations of local field potentials and in Current Source Density analysis

Authors: *D. K. WOJCIK[1], T. B. NESS[3], C. H. CHINTALURI[2], J. POTWOROWSKI[2], H. GLABSKA[2], S. LESKI[2], G. T. EINEVOLL[3];
[2]Dept. of Neurophysiol., [1]Nencki Inst. of Exptl. Biol., Warszawa, Poland; [3]Dept. of Mathematical Sci. and Technol., Norwegian Univ. of Life Sci., Aas, Norway

Abstract: Multielectrode recordings of local field potentials (LFP) from brain slices are a standard research technique. To test different methods of LFP analysis we need to have realistic ground truth data which demands plausible models of neural activity and taking into account physical properties of the setup, tissue, and the electrodes. To decorrelate the electrode signals it is often useful to reconstruct the Current Source Density from the measured potentials (CSD). In CSD analysis one often assumes field propagation in isotropic and homogeneous tissue, which is not true in slice recordings.
We studied the effect of realistic conductivity profiles and the slice geometry on i) computation of LFP generated by cell populations embedded in slice, as would be measured on MEA, and ii) CSD reconstruction in the slice from such potentials.
First, we investigated how the use of increasingly detailed physical models of slice tissue affects the resulting model LFPs. We simulated Traub's model of thalamo-cortical loop (Traub et al. (2005)) in NEURON and the extracellular potentials in uniform, homogeneous medium were computed post-hoc from tracked trans-membrane currents. To verify the need for inclusion of experimental setup context we modeled the field in the tissue and saline using finite-element approach (FEM). The slice was placed in a saline solution, the cortical column was put inside the slice, and we computed the extracellular potentials it generated at the electrode plane underneath. We saw that the inclusion of slice setup noticeably modifies the observed activity as both the amplitude and shape of the potential profile were changed. However, inclusion of inhomogeneity

and anisotropy in the computations does not lead to substantial changes of the profile and amplitude. Indeed, inaccurate estimation of conductivity will in general introduce bigger errors than assuming homogeneous and isotropic tissue.
We also obtained an approximation to the potential computed at the electrode grid from the slice using the method of images (MOI). In computation of the LFP for the cases studied, the MOI approximation gives results very close to FEM model while being much more efficient computationally. To improve CSD estimation in the slice we included MOI model in the kernel CSD method. Testing the new kCSD variant on FEM model data we found that i) the reconstructed error is smaller when the correct slice thickness and the difference in conductivities (slice/saline) are taken into account, but the improvement is relatively minor, ii) the first two extra terms from the method of images are enough.

Disclosures: D.K. Wojcik: None. **C.H. Chintaluri:** None. **S. Leski:** None. **J. Potworowski:** None. **H. Glabska:** None. **G.T. Einevoll:** None. **T.B. Ness:** None.

Poster

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.04/NNN34

Topic: G.06. Computation, Modeling, and Simulation

Title: Fornix deep brain stimulation induces functional activation in hippocampal circuitry

Authors: *E. K. ROSS[1,2], J. KIM[2], S. HAN[2], P. MIN[2], K. LEE[2,3];
[1]Neurosci., Mayo Clin. Col. of Med., Rochester, MN; [2]Neurosurg., [3]Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Deep brain stimulation (DBS) has emerged as a treatment for various neurologic and neuropsychiatric disorders, including Parkinson's disease, dystonia, depression, and obsessive-compulsive disorder (Lang et al., 1998). Though the mechanisms behind therapeutic benefit of stimulation-induced neuromodulation are not completely understood, it has been established that modification of brain function by DBS depends on targeting specific sites in the complex underlying neuronal circuitry (Lee et al., 2009). Alzheimer's Disease (AD), among many other forms of dementia, is characterized by progressive deterioration of memory and other cognitive functions (Querfurth et al., 2010). Recently, DBS has been applied to the Papez circuit to address the memory function associated with dementia (Laxton et al., 2010; Suthana et al., 2012). As part of the Papez circuit, the fornix is thought to play an integral role in declarative memory. DBS within the medial temporal lobe at the level of the fornix is a promising approach to addressing

memory deterioration associated with dimentia. In this study, we address functional connectivity of fornix stimulation, as measured by fMRI before and after chronic stimulation, in a DBS large animal model (pig) using the same stimulation parameters and electrode configuration currently applied in human patients. We conducted a preliminary study of DBS of the fornix and found activation in the hippocampus, entorhinal cortex, parahippocampal gyrus, substantia nigra, nucleus accumbens, and prefrontal cortex. In this study, we show that this activation is voltage-dependent. Finally, using FSCV we show that fornix DBS results in dopamine release in the nucleus accumbens (NAc), which implicates this circuitry in hippocampal-based declarative memory. In previous studies, structural MRI, and PET scan in early AD revealed hippocampal atrophy and cingulum bundle disruption was highly correlated with posterior hypometabolism, which is hallmark in AD (Villain et al., 2008). Sexton et al. reported that episodic memory was associated with hippocampal volume and mean diffusivity of cingulum and fornix (Sexton et al., 2010). Additionally, it has been reported that age-related decline in recall was specifically associated with degradation of fornix microstructure, consistent with the view that this tract is important for episodic memory (Metzler-Baddely et al., 2011). Taken together, these data support the idea that DBS within the medial temporal lobe at the level of the Papez circuit shows promise for memory enhancement.

Disclosures: E.K. Ross: None. **J. Kim:** None. **S. Han:** None. **P. Min:** None. **K. Lee:** None.

Poster

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.05/NNN35

Topic: G.06. Computation, Modeling, and Simulation

Support: DOD Spinal Cord Injury Research Program (SCIRP) under contract number W81XWH-11-1-0819

Title: Simulation analysis of conduction block in unmyelinated axon after high-frequency electrical stimulation

Authors: *G. YANG[1,3], J. WANG[1], J. R. ROPPOLO[2], W. C. DE GROAT[2], C. TAI[1]; [2]Dept. of Pharmacol. and Chem. Biol., [1]Univ. of Pittsburgh, Pittsburgh, PA; [3]Dept. of Biomed. Engin., Beijing Jiaotong Univ., Beijing, China

Abstract: Nerve conduction block induced by high frequency (>5 kHz) electrical stimulation have many potential clinical applications. Although the original Hodgkin-Huxley model can successfully simulate the conduction block in unmyelinated axon during the stimulation, it failed

to simulate the conduction block during the period after the stimulation. In this study, the Hodgkin-Huxley model was modified to include an electrogenic sodium-potassium pump and successfully simulated the post-stimulation conduction block. Simulation analysis indicates that high frequency electrical stimulation causes continuous sodium influx and increases intra-axonal sodium concentration that can only recover slowly after the stimulation, causing conduction block during the post-stimulation period. The duration of the post-stimulation block is proportional to the duration and intensity of high frequency electrical stimulation. Understanding the mechanisms underlying high frequency block is important to further promote its clinical applications.

Disclosures: G. Yang: None. **J. Wang:** None. **J.R. Roppolo:** None. **W.C. de Groat:** None. **C. Tai:** None.

Poster

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.06/NNN36

Topic: G.06. Computation, Modeling, and Simulation

Support: Centre National de la Recherche Scientifique (CNRS, France)

Agence Nationale de la Recherche, Complex-V1 (ANR, France)

European Community (BrainScales, Magnetrodes)

NIH NS18741

NIH EB009282

NIH NS44623

Title: Frequency scaling properties of EEG and MEG signals at high frequencies are consistent with diffusive electric properties of intracellular and extracellular media

Authors: *N. DEHGHANI[1,2], C. BEDARD[2], S. CASH[3], E. HALGREN[4], A. DESTEXHE[2]; [1]Wyss Inst., Harvard Univ., Boston, MA; [2]Lab. for Computat. Neuroscience. Unité de Neurosciences, Information & Complexité (UNIC), Ctr. Natl. de la Recherche Scientifique (CNRS), Gif-sur-yvette, France; [3]Dept. of Neurology, MGH, Harvard Med. School., Boston, MA; [4]Multimodal Imaging Lab, Dept. Radiology and Neurosciences, Univ. of Californica, San Diego (UCSD), La Jolla, CA

Abstract: Electro-encephalogram (EEG) and magneto-encephalogram (EEG) signals can be recorded simultaneously and provide two complementary signals of neuronal activity. It was shown previously that EEG and MEG signals display different frequency-scaling properties for low frequencies (Dehghani et al., JCNS 2010). Theoretical models show that if the extracellular medium is resistive, then the scaling exponent at low frequencies should be the same for EEG and MEG signals. Therefore, the different exponents observed constitute an indirect evidence for non-resistive extracellular properties. Here, we complement this study by providing a frequency-scaling analysis for high frequencies. We show theoretically that if the extracellular and intracellular media have diffusive electrical properties, then EEG and MEG signals should scale identically for high-frequencies (>20 Hz).Analysis of simultaneous EEG and MEG signals for 4 different subjects show that this is indeed the case, suggesting that diffusive properties are important. Diffusive extracellular media are also consistent with other measurements such as the frequency scaling of local-field potential (LFP) signals, or the transfer function between intracellular and extracellular potentials. In a companion poster (Bedard et al., this conference) we show that extracellular impedance measurements are indeed compatible with the paricipation of diffusive properties. We conclude that the diffusive properties of intracellular and extracellular media provide a framework that explains a large body of direct and indirect experimental measurements.
Ref: Dehghani N, Bédard C, Cash SS, Halgren E, Destexhe A. Comparative power spectral analysis of simultaneous elecroencephalographic and magnetoencephalographic recordings in humans suggests non-resistive extracellular media. J Comput Neurosci. 2010;29(3):405-21.

Disclosures: N. Dehghani: None. **C. Bedard:** None. **S. Cash:** None. **E. Halgren:** None. **A. Destexhe:** None.

Poster

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.07/NNN37

Topic: G.06. Computation, Modeling, and Simulation

Support: Loyola Research Support Grant

Title: Impact of surface charges on cellular mechanics in a uniform DC electric field - A model study

Authors: *H. YE;
Dept. of Biol., Chicago, IL

Abstract: Cells deform and migrate under electric field, partially mediated by the interactions between the field and the electric charges on the cell membrane. Two kinds of charges present on the cell membrane: the intrinsic charges carried by the charged proteins, and the free charges induced by the electric field. How do these surface charges involve in the biomechanics of the cells that contribute to the membrane deformation and migration of the cell? Using a simple spherical cell model, we computed the distribution of the electric fields, the induced surface charges, the forces and torques exerted on the cell. We found that the biomechanical roles of the two charge groups are different -while the induced charges could be involved in membrane deformation in the DC field, the intrinsic charges could contribute to cell migration. Shape deformation depends on the ratio between the conductivities of the medium and the cytoplasm (Figure 1). Translational force generated on the cell is proportional to the net intrinsic charges carried by the cell membrane (Figure 2). The calculated forces for deformation and migration are both quantitatively comparable to those reported in the literature. It is therefore possible to control cell mobility and deformation by manipulating different charge groups.

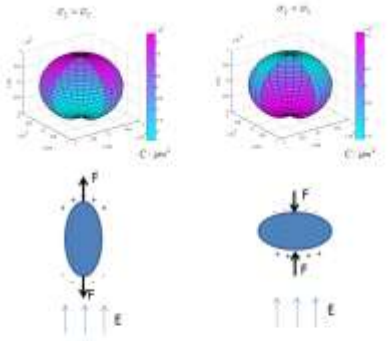

Figure 1. Induced electric charges on the cell and its involvement in cell deformation.

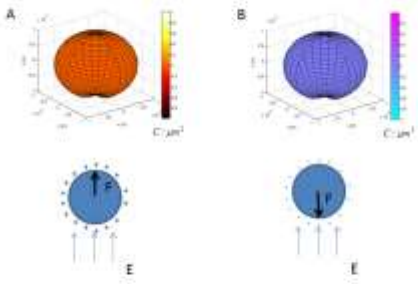

Figure 2. Intrinsic electric charges on the cell and its involvement in cell migration.

Disclosures: H. Ye: None.

Poster

587. Computation, Modeling, and Simulation VII

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.08/NNN38

Topic: G.06. Computation, Modeling, and Simulation

Support: 2R01NS047293

Title: EEG cortical patch sources and equivalent dipole source localization

Authors: *Z. AKALIN ACAR[1], S. MAKEIG[2];
[1]Inst. for Neural Computation, Univ. of California San Diego, LA JOLLA, CA; [2]Univ. of California San Diego, La Jolla, CA

Abstract: Accurate of EEG source localization requires a realistic electrical forward model of the subject's head based on a structural head model, and a realistic source model when solving the inverse problem. In linear source localization of EEG, the most commonly used anatomical constraint on source distribution is to restrict the dipoles to the cortical surface with fixed orientations perpendicular to the surface. The magnitudes are then allowed to change during a given experiment. This is based on the fact that the neuronal electric activity that can be measured outside the skull by EEG and MEG originates primarily from groups of neurons organized in macro-columns perpendicular to the surface of the cortex [1]. Taking this a step further, a physiologically relevant assumption is to construct the source space using “patches” that are tangential to the surface, based on the observation that metabolically active areas are closely related to active neuron groups. We have shown the use of patch-based electro-cortical source localization in our previous studies [2]. In this work, we present a comparison of dipolar and patch-based source models and investigate source localization errors when a simulated recording of a patch-based source is localized using a dipolar source.
We used a subject-specific four-layer realistic head model and simulated EEG for 8,000 locations on the subject’s cortical surface. The EEG scalp maps were calculated for three Gaussian-tapered patches with geodesic radii of 10 mm, 6 mm, and 3 mm. For each patch activity equivalent dipole source localization was performed using the same head model, a four-layer template MNI head model, and a spherical head model.
When the patch size was small the source can be better modeled as a point source, so the error magnitudes were lowest for 3 mm radius patches. For larger patches, the dipole was localized farther away, below the patch, so the localization errors increased. However, with the realistic head model, the maximum error was 2 mm. The maximum error for the MNI model was 8 mm, and the errors increased up to 2 cm for the spherical model. The mean orientation errors obtained

by 10 mm patches were 0.7 degrees using the realistic model and 6.7 degrees using the other head models. The errors for the equivalent dipoles fitted using the realistic model is below 2 mm, which suggests that a dipole source model is suitable for most purposes.

References:
1.Baillet, Mosher, Leahy, 'Electromagnetic brain mapping', IEEE Signal Proc. Magazine, 18(6), 2001.
2.Akalin Acar et al, 'Electrocortical source imaging of intracranial EEG data in epilepsy', IEEE EMBC, 2011.

Disclosures: Z. Akalin Acar: None. **S. Makeig:** None.

Poster

587. **Computation, Modeling, and Simulation VII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 587.09/NNN39

Topic: G.06. Computation, Modeling, and Simulation

Support: Wallace Coulter Foundation

DoD

Title: Spatial and polarity precision of high-definition transcranial direct current stimulation (HD-tDCS)

Authors: M. ALAM, *M. BIKSON, D. TRUONG;
Dept of Biomed. Engin., City Col. of New York, NEW YORK, NY

Abstract: Transcranial Direct Current Stimulation (tDCS) is a non-invasive neuromodulation technique that applies low amplitude current via electrodes placed on the scalp. Rather than directly eliciting a neuronal response, tDCS is believed to modulate excitability - encouraging or suppressing activity in regions of the brain depending on the polarity of stimulation. Studies have explored a wide range of clinical applications including neuropathic pain, motor rehabilitation, speech rehabilitation, working memory as well as others. In these cases, the particular application of tDCS is determined by the electrode configuration and intensity of stimulation. Relatively large (5x7 cm) conventional sponge pads in a 1 anode and 1 cathode (1x1) montage have been the standard configuration used in past studies. In recent years the 4x1 High-Definition (HD) tDCS ring configuration has been explored as an alternative montage with improved spatial control. The extent of this control was explored through MRI-derived Finite Element (FE) models.

Finite element models were created to analyze the cortical electric field generated during tDCS. High resolution MRIs were segmented into seven tissue/material masks of varying conductivities through a combination of automated and manual tools. Computer generated models of electrodes, gel, and/or sponge pads were incorporated into the segmentation. Volume meshes were generated, boundary conditions were applied, and the Laplace equation ($\nabla\cdot(\sigma\nabla V)= 0$) was solved. The resulting cortical electric field was interpreted as a correlate for stimulation and modulation.
In comparison to conventional sponge pads 4x1 HD-tDCS has a number of advantages, the most pronounced being focality. Studies with a mechanistic, anatomical, emphasis could be better served by this more precise method of stimulation that could avoid potentially confounding brain regions. Still, conventional pad stimulation has an extensive history of use. Clinicians may feel comfortable relying on the established safety record of conventional pads, but modeling results suggest much more spatial and polarity control utilizing the 4x1 configuration. This degree of control can be leveraged in cases where an additional safety factor would be warranted, particularly in susceptible populations (e.g. implants or skull defects). As more is understood about the brain and how different regions may affect clinical outcomes, 4x1 HD-tDCS can provide both the spatial and polarity precision needed to target these regions.

Disclosures: M. Alam: None. **M. Bikson:** None. **D. Truong:** None.

588**Poster**

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.01/NNN40

Topic: G.06. Computation, Modeling, and Simulation

Support: DFG SFB TR 31

Title: Fast and reliable estimation of non-Gaussian stimulus receptive fields using large-margin classification

Authors: *A. F. MEYER[1], J.-P. DIEPENBROCK[2], F. OHL[2], J. ANEMÜLLER[1];
[1]Physics, Carl Von Ossietzky Univ. Oldenburg, Oldenburg, Germany; [2]Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: The receptive field (RF) represents a classic approach to the characterization of sensory neurons through a linear filter. The underlying neuronal model consists of a linear non-linear cascade model that drives a spike-generating Poisson process [Chichilnisky 2001]. When computed with stimuli that are non-white or non-Gaussian, in particular natural stimuli, standard

RF estimation methods like the spike-triggered average (STA) and variations thereof have been shown to result in biased estimates of the true receptive field.
Recently developed RF estimation methods include semi-parametric information theoretic approaches that in theory allow for unbiased RF estimates independent of stimulus statistics and neural response nonlinearity [Sharpee et al. 2004]. However, numerical optimization of the underlying non-convex objective functions is non-trivial in practice. It results in comparably high variance of obtained RF estimates, in particular when available data is of limited length.
The approach pursued here assumes the well-known neuron model of a weighted linear integrator with a noisy threshold operation, which together determine the spike/non-spike response. In stimulus input space, a hyperplane defined by linear filter and threshold value optimally separates spike-eliciting stimulus examples from non-spike-eliciting ones.
We propose to estimate this optimal hyperplane using a modified largin-margin classifier and show that this is equivalent to estimating the neuron's linear receptive field. In contrast to STA-based estimators, the approach does not make any assumptions regarding multivariate stimulus distribution or correlation structure of the stimulus ensemble. In contrast to information theoretic approaches, optimization is performed on a well-behaved convex goal function. The resulting method bears qualitative similarities to maximization of mutual information between stimulus and response while maintaining the advantage of convex optimization.
Using simulated responses, we demonstrate that the proposed approach is robust to highly non-Gaussian stimulus ensembles. Findings are verified by estimation of spectro-temporal receptive fields (STRFs) for the inferior colliculus of mongolian gerbils, recorded in response to auditory stimuli. In the large data regime, the presented approach performs equal to unbiased information-theoretic estimators. With limited amounts of data, it exhibits significantly smaller variance of the obtained RF estimates. The method may permit more accurate investigation of neural effects such as adaptation that occur on smaller time scales than common RF estimators require to converge.

Disclosures: A.F. Meyer: None. **J. Anemüller:** None. **J. Diepenbrock:** None. **F. Ohl:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.02/NNN41

Topic: G.06. Computation, Modeling, and Simulation

Support: The Si elegans project 601215 is funded by the 7th Framework Programme (FP7) of the European Union under FET Proactive, call ICT-2011.9.11: Neuro-Bio-Inspired Systems (NBIS).

Title: Introducing the Si elegans project - The quest for understanding, emulating and reverse-engineering nervous system function in Caenorhabditis elegans

Authors: *A. BLAU[1], A. DE MAURO[3], E. DI FABRIZIO[2], G. EPELDE[3], C. LIBERALE[2], G. MACLAIR[3], M. MCGINNITY[4], F. MORGAN[5], V. RAJAMANICKAM[2];
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Abstract: One of the simplest nervous systems in nature - that of the hermaphrodite of the nematode *Caenorhabditis elegans* - still evades our complete understanding. Despite being one of the five best characterized model organisms with all of its 302 neurons and almost its entire connectome precisely mapped, there is only sparse knowledge on how its nervous system codes for its rich behavioral repertoire. The European ***Si elegans*** project aims at unravelling *C. elegans*‘ nervous system function by emulating it with 302 parallelly interconnected field-programmable gate array (FPGA) neurons and by embodying this hardware nervous system with a biophysically accurate soft-body representation in a virtual behavioral arena. In a closed-feedback loop, any sensory experience of the virtual nematode in its virtual environment will be processed by sensory and subsequently interconnected neurons to result in motor commands at neuromuscular junctions at the hardware-software interface to actuate virtual muscles of the virtual nematode. The postural change in the virtual world will lead to a new sensory experience, thereby altering the set of virtual stimuli to be processed by the hardware nervous system and thus closing the loop. We will present the overall concepts, first implementation steps and our envisioned reach-out strategies to involve the neuroscience community at large in an open-source and peer-contribution spirit. For further information and recent news please visit www.si-elegans.eu.
Acknowledgements: This project 601215 is funded by the 7th Framework Programme (FP7) of the European Union under FET Proactive, call ICT-2011.9.11: Neuro-Bio-Inspired Systems (NBIS).

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Disclosures: A. Blau: None. **A. De Mauro:** None. **E. Di Fabrizio:** None. **G. Epelde:** None. **C. Liberale:** None. **G. Maclair:** None. **M. McGinnity:** None. **F. Morgan:** None. **V. Rajamanickam:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.03/NNN42

Topic: G.06. Computation, Modeling, and Simulation

Support: FP7 European Commission NoE n257462 HYCON2

Title: Analytical conditions for the entrainment of basal ganglia structures by their exogenous inputs

Authors: *A. CHAILLET;
L2S - Supélec, Gif Sur Yvette, France

Abstract: Due to the nonlinear nature of the dynamics involved, the oscillations generated in a neuronal population may occur at a different frequency from the stimulus that generated it. An illustrative example of this fact is the Wilson & Cowan model, consisting of the coupling between two neuronal populations involving inhibitory and excitatory neuron models: depending on its parameters, this model may for instance exhibit sustained oscillations in response to a constant stimulus.
A tool developed in the control theory community offers a systematic way to guarantee that a nonlinear dynamical system can be entrained by its periodic input, meaning that it eventually tends to a rhythmic behavior at the input's frequency. This tool is known as contraction, convergence, or incremental stability and constitutes a noteworthy way to check whether a

network of neuronal populations will be entrained by its input, or rather exhibit endogenous frequencies. Several techniques can be used to establish this feature in practice; in particular, we have recently introduced a Ratzumikhin-type condition to ensure incremental stability in presence of delays (Chaillet et al. 2013).
Here, we show how this theoretical framework can help in analyzing the basal ganglia oscillations in Parkinson's disease (PD). Several works have studied the possible pacemaker role played by the interaction between the subthalamic nucleus (STN) and the global pallidus pars externa (GPe) in the generation of the beta-band oscillations characterizing PD. Some of these works rely on a delayed version of the Wilson & Cowan model (Nevado-Holgado et al. 2010, Pasillas-Lépine 2012, Pavlides et al. 2012, Haidar et al. 2013). An alternative hypothesis suggests that these pathological beta-oscillations may be endogenously generated by the striatum and then propagate to the basal ganglia network by an entrainment mechanism (McCarthy et al. 2011). The analysis we conduct here provides easily checkable conditions on the interconnection weights between the individual populations and the slope of their activation functions for the STN-GPe network to be incrementally stable, in which case it may indeed be entrained by a striatum endogenous beta-oscillation.

Disclosure.DisclosureBlock:

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.04/NNN43

Topic: G.06. Computation, Modeling, and Simulation

Title: A neural coding scheme to allow global workspace functionality

Authors: *K. J. HAYWORTH;
Howard Hughes Med. Inst., Ashburn, VA

Abstract: Two basic operations performed in a computer are copying information from one register to another and comparing two registers for equality. These operations underlie the computer's ability to create and manipulate structured representations. Models of the human cognitive architecture (e.g. SOAR, ACT-R, GWT) posit that the brain must also be capable of these two operations -positing a global workspace of buffers among which copies and comparisons can be performed. Progress has been made in explaining how such buffers might be implemented in the brain. For example, the visual hierarchy ends in several cortical buffers

representing an object's shape, color, and motion as rate-coded firing patterns. However, there is little understanding of how the contents of these buffers are copied or compared. In simple stimulus-response tasks such copying and comparison may be unnecessary, but when modeling even mildly more complicated tasks such operations have proved indispensable as discussions of the "neural binding problem" make clear (Malsburg 1999). I will present a neural model, the Dynamically Partitionable AutoAssociative Network (DPAAN), for how such operations might be performed. The key to this model is the realization that the brain's coding strategy cannot be similar to that which a computer uses (i.e. a unique pattern of active bits designating each symbol in the computer's vocabulary). Such an engineered solution relies on one-to-one wiring which is not biologically plausible. Instead, the DPAAN model posits that each symbol is actually coded differently in each of the brain's buffers, but that each of these different codings is related by each being a particular piece of the same "global" attractor state. In the DPAAN model, a set of switchable synapses connect the brain's buffers and switching these on effectively merges all buffers into one global autoassociative network which can be trained to have a set of stable states -each one representing one symbol in the brain's vocabulary. Switching off these connections reduces the global network to a set of independent buffers with, crucially, each still retaining the full complement of trained stable states. Copying information from buffer A to buffer B simply entails switching on the synapses projecting from A to B. Comparing the informational contents of A and B requires determining if the firing patterns in the two are part of the same global attractor state. Simulations will be presented demonstrating how this neural coding strategy can be used to perform visual attribute binding and rule-based operations, and how a DPAAN can be used as a neural implementation of ACT-R's global workspace.

Disclosures: K.J. Hayworth: None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.05/NNN44

Topic: G.06. Computation, Modeling, and Simulation

Support: NSF Center for the Science of Information Grant CCF-0939370

NEI Vision Grant EY019684

Title: Modeling hierarchical visual computation with the Matlab environment for deep architecture learning (MEDAL)

Authors: *D. E. STANSBURY[1], J. L. GALLANT[2];
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Abstract: Current computational models of neural processing account for many of the properties exhibited by neurons in peripheral sensory areas, but they have had less success in later stages of the perceptual hierarchy. The anatomical organization of the brain suggests that these later stages of processing can be modeled as a transformation of sensory inputs across a series of hierarchically-organized, nonlinear computations. A modeling approach that reflects this organization might provide a powerful tool for modeling intermediate stages of processing. Recent advances in machine learning have produced learning algorithms and model architectures that model data through multiple nonlinear layers. These methods, known as deep architecture learning (DAL), offer the neuroscience community a promising approach for improving models of neural computation. To facilitate the application of DAL methods for computational neuroscience, we have developed the Matlab® Environment for Deep Architecture Learning (MEDAL). The MEDAL software suite compiles many state-of-the-art DAL algorithms into an object-oriented framework that is easy to use. The MEDAL suite provides many routines for stimulus preprocessing, a vast array of model architectures, and a variety of model visualization tools appropriate for neuroscience applications. We demonstrate the functionality of the MEDAL software suite by modeling neurons in both the feline and primate visual systems. We find that initializing model weights using unsupervised learning greatly improves model accuracy when training data is limited. We also find that submodules available in the MEDAL suite can account for simple and complex cell behavior in V1. Finally, we find that deep model architectures provide more accurate models of intermediate stages of visual processing (V2 and V4) than can be obtained using shallow computational models commonly used in the field.

Disclosures: D.E. Stansbury: None. **J.L. Gallant:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.06/NNN45

Topic: G.06. Computation, Modeling, and Simulation

Title: A NetLogo model of the Notch regulatory network in the determination of developmental patterning

Authors: R. HIMMELWRIGHT[1], J. PFAFFMANN[2], *E. R. REYNOLDS[1];
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Abstract: The *Notch* (N) signaling pathway is involved in cell fate decision and developmental patterning in diverse organisms. We have developed an Agent-Based model of this pathway as characterized in the early *Drosophila* embryo that allows for a representation of individual molecules, their movement and their interactions within a modeling environment. We are using the model to investigate how specific molecular components that stabilize fate at the cellular level also are involved the formation of pattern in the larger group of cells. Currently our model represents many of the major molecular components of the pathway and allows us to control the levels of these components, their transition from one state to another and their movement from the nucleus to the cell membrane and back. Most steps introduce randomness into the system using probabilities of events rather sequential direct implementation.

A data logging mechanism captures an integer count of cells with a neuronal fate (as defined as a lack of *N* product) at each time step and stores them to a text file as a number sequence that preserves cell position, allowing data to be analyzed both quantitatively and spatially. Using a plot of neuron count vs. time of either single run or aggregate data, a linear regression can be applied to different regions of the graph to track data trends of the model run. Regression analysis of the peak and trough data characterizes the shape of the oscillations, while the intercept from the general regression gives a reasonable approximation of where the system is stabilizing. To quantitate pattern, we use a modification of Hamming distance that measures amount of dis-similarity between successive runs at successive spatial positions within the model.

With a wide set of initial parameters, our current model can accurately reproduce the rosette pattern of neurons and skin cells in the system through oscillations in cell fate that settle into the final fate pattern. Our first experiments define the role of specific molecular events in formation of this stable pattern. We have explored the roles of initial transcriptional rates, processing of *Dl*, processing of *N* during and after cleavage, as well *N* regulation of its own transcription and that of *Dl*. In general, results indicate that initial transcription rate can be varied and a stable pattern is still obtained. However, *Dl* processing, autoregulation of *N* transcription, and the timing of *N* cleavage with transport to the nucleus do affect the model's ability to obtain a stable and correct pattern of fate determination.

Disclosures: R. Himmelwright: None. **E.R. Reynolds:** None. **J. Pfaffmann:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.07/NNN46

Topic: G.06. Computation, Modeling, and Simulation

Title: Machine intelligence and learning in avionic systems

Authors: *H. C. YUAN;
Raytheon, El Segundo, CA

Abstract: Airborne avionic systems employ sensors, such as radar and infrared devices, which receive electromagnetic and optical signals. These analog signals are processed through amplification, detection, filtering and demodulation, then the signals are sampled into digital data streams and flow into a high speed digital processor. Modern airborne digital processors are programmed in high level languages with algorithms processing digital data to make real time decisions. The digital processor is the "brain" of these avionic systems, making decisions, controlling all the functions of the systems, and the algorithms implemented in the processor comprise a "machine intelligence and learning" system.
The aim of machine intelligence and learning in such systems is to supply "situational awareness" information to the avionics operator. To provide this "situational awareness", the data and information from these sensors is processed so that many decisions can be made in fractions of seconds. The operator of these systems can respond and make other decisions according to the processed information.
Research in machine intelligence, as illustrated by the research of Eliezer Yudkowsky (Cognitive Biases Potentially Affecting Judgment of Global Risk - 2008; Levels of Organization in General Intelligence - 2007), has been evolving with increasingly complex models of the world at a global level and individual or macro systems at the organizational level. In avionic systems, machine intelligence and learning models have become more sophisticated and complex with the increases in memory capacity and speed of modern digital processors, and with the application of parallel processing architectures. Airborne avionic learning systems also employ fuzzy logic intelligence models in their algorithms. The fuzzy logic models add a dimension to "situational awareness" by handling sensor data or information that is in conflict with current data, is unknown or inconsistent with current information. Fuzzy logic also adds dimension by managing sensor data according to membership functions for intentional or pertinent information vs. unintentional (random) or confusing information.
This poster presents an overview of how fuzzy logic models are applied as machine intelligence to airborne avionic systems and explores how these models can be applied to research in neuroscience.
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Disclosures: H.C. Yuan: None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.08/NNN47

Topic: G.06. Computation, Modeling, and Simulation

Title: Are you smarter than a mouse?

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Abstract: Maze learning has been used as a measure of spatial intelligence for decades. The Hebb-Williams maze, a type of visuo-spatial task, consist of twelve standardized problems that vary based on difficulty level, as well as the type of learning and memory needed to correctly solve the maze. These mazes have been used to examine spatial learning abilities across a wide range of species, including rats, cats, rabbits, ferrets, mice, monkeys, and even humans. Research has demonstrated that human performance on a virtual Hebb-Williams maze is statistical similar to problem solving skills employed by mice (Shore et al. 2001). More recently a study demonstrated that performance on a virtual Hebb-Williams (HW) maze by individuals affected by Fragile X Syndrome was similar to animal models of the disorder using a physical version of the HW mazes (McLeod et al. 2009). However it is unclear whether different virtual environments will affect human performance on this maze task and therefore influence the ability to translate the results generated between humans and animal models. Our work examines the performance of children (ages 8-12) and adults (ages 18-22) on a virtual Hebb-Williams maze task utilizing different virtual environments. Our study examines the influence of different graphical representations of the HW mazes on performance in a virtual environment in comparison to C57BL/6J mice completing a physical version of the HW mazes. Our data suggest that the virtual environment may influence performance efficiency on this task and therefore our ability to translate findings between species.

Disclosures: J.F. Cysner: None. **M. Manglani:** None. **N. Escalona:** None. **M. Taylor:** None. **E. Johnson:** None. **L.A. Gabel:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.09/NNN48

Topic: G.06. Computation, Modeling, and Simulation

Title: SCRalyze - a toolbox for inferring sympathetic arousal from physiological recordings

Authors: *D. R. BACH[1,2], R. J. DOLAN[2], K. J. FRISTON[2];
[1]Zurich Univ. Hosp. For Psychiatry, Zurich, Switzerland; [2]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstract: Observed animal or human behaviour is often used to operationalize central - psychological or neural - states. Here, we propose to formalize operational definitions as explicit causal models. These can be used to infer hidden states. When probabilistic models are used, this approach can increase precision in inferring central states, compared to operational approaches. A common human example is inference on sympathetic arousal (SA) from observed skin conductance responses (SCR). Here, we propose and validate a model-based approach to SCR by formalising a peripheral system that can be approximated as linear and time-invariant, and creating various neural models for different experimental situations. We show that for several classes of experiments, SA estimates yield a more precise prediction of the (known) central process than raw SCR values. A Matlab-based software suite - SCRalyze - implements this approach and provides for extension to other physiological, or behavioural, measures. We propose that causal models are a powerful tool to infer central processes from observed behaviour.

Disclosures: D.R. Bach: None. **R.J. Dolan:** None. **K.J. Friston:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.10/NNN49

Topic: G.06. Computation, Modeling, and Simulation

Support: NSF IGERT Training Program

SNF grant 320030-130090

Spanish Government grant E-28-2012-0504681

Leenards Foundation

JS McDonnell Foundation

Title: Multi-scale community organization of the human structural connectome and its relationship with resting-state functional connectivity

Authors: *R. F. BETZEL[1,2], A. GRIFFA[3,4], A. AVENA-KOENIGSBERGER[1,2], J. GOÑI[1], J.-P. THIRAN[3,4], P. HAGMANN[3,4], O. SPORNS[1,2];
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[4]Dept. of Radiology, Univ. Hosp. Ctr. and Univ. of Lausanne, Lausanne, Switzerland

Abstract: The human connectome is defined as the human brain's full set of neural elements and their anatomical connections and it has been extensively mapped and widely studied at different spatial resolutions over the past several years, particularly in the cerebral cortex. A principal finding is that the cortical connectome can be decomposed into network communities of densely interconnected brain regions. Such structural communities, in turn, are often conceptualized as modules underlying different aspects of brain function. The process of community detection, however, may be subject to methodological limitations. For example, numerous studies attempting to infer the connectome's community structure have used a modularity measure that is prone to fail when communities range over many different sizes or when communities are forming a nested hierarchy. Also, these studies relied on the intuition that community structure is best defined in terms of a network's static topology as opposed to a definition that makes reference to dynamic processes unfolding within the structural network. In the present study we used the partition stability framework, which defines communities in terms of a Markov process (random walk), to infer the connectome's community structure at multiple dynamical scales. The connectome's multi-scale community structure was compared to observed resting-state functional connectivity, which is defined by the magnitude of statistical dependence between blood oxygen-level dependent (BOLD) signals recorded from different brain regions. Resting state functional connectivity is also believed to reflect communication and integration of information between brain regions. This comparison revealed communities across a broad range of dynamical scales that bore close resemblance to observed patterns of functional connectivity. This result suggests a relationship between communities in structural networks, models of communication processes, and brain function. It further suggests that communication in the brain is not limited to a single characteristic temporal scale, leading us to posit a heuristic for scale-selective communication in the cerebral cortex.

Disclosures: R.F. Betzel: None. **O. Sporns:** None. **A. Avena-Koenigsberger:** None. **J. Goñi:** None. **A. Griffa:** None. **P. Hagmann:** None. **J. Thiran:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.11/NNN50

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Grant R01HD051747

Title: Precision matters in gpu based eeg/meg forward solution

Authors: *N. B. BANGERA, J. D. LEWINE;
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Abstract: Forward solution is a critical component in source localizations methods utilized for finding epileptogenic foci. Based on the complexity of the model used, the calculation of the forward solution can get very computationally intensive. The processing power of the graphical processing units (GPU) on commercially available video cards can now be harnessed very easily using parallel programming platforms such as NVIDIA CUDA to solve such computationally intensive methods in a fraction of time required by the CPU. Our previous work translated algorithms from the CPU to the GPU domain using CUDA for computing both the Spherical and Boundary Element Model (BEM) based forward solution for EEG and MEG. The same inputs will output the same results up to a given floating point precision on the CPU and GPU. Hence differences between the CPU and GPU calculated forward solution need to be interpreted carefully. Also, older generations of GPU do not support calculations in double precision. In this study, we investigate the impact of floating point precision and verify whether single precision or double precision calculations are adequate for GPU based E/MEG forward solution for the spherical and BEM model.

For the comparison between the CPU and GPU solution, electric field and magnetic field at 64 and 306 sensors respectively was calculated on the CPU in double precision for known dipole locations in two model types (spherical and BEM) and used as the gold standard reference solution. The difference between the GPU and CPU solution was measured using L2 norm. In addition, we verified the GPU results using a beamformer based inverse solution. Firstly, we simulated EEG and MEG data for dipoles at random locations using a CPU solution in double precision. The inverse solution was then calculated using the leadfields calculated using the GPU in both single and double precision and tested against the actual dipole locations. Thus, the GPU forward solution is tested against the CPU solution using both a direct comparison as well an inverse based approach.

The single precision GPU solution for EEG was found to be accurate for both the spherical and BEM models. However, single precision GPU solution for MEG led to incorrect localization of dipole locations in both models. MEG forward solution calculated in double precision on the GPU resulted in accurate source localizations. Our results thus show that CUDA based GPU solution for MEG requires hardware with support for double precision computation whereas EEG solution can be safely computed on single precision NVIDIA GPU.

Disclosures: N.B. Bangera: None. **J.D. Lewine:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.12/NNN51

Topic: G.06. Computation, Modeling, and Simulation

Support: National Science Foundation CAREER award IOS - 1054914

National Institute of Mental Health grants MH065561 and MH073057

SURF undergraduate grant from the College of Charleston

HHMI Undergraduate Fellowship

Title: What is all the noise about in interval timing?

Authors: *P. G. LYNN[1], D. NOVO[2], S. OPRISAN[2], C. BUHUSI[3];
[2]Physics and Astronomy, [1]Col. of Charleston, Charleston, SC; [3]Psychology, Utah State Univ., Logan, UT

Abstract: The perception and use of durations in the seconds-to-hours range (interval timing) is essential for survival and adaptation, and is critical for fundamental cognitive processes like decision making, rate calculation, and planning of action. The classic interval timing paradigm is the fixed-interval (FI) procedure in which a subject's behavior is reinforced for the first response (e.g., lever press) made after a pre-programmed interval has elapsed since the previous reinforcement. Subjects trained on the FI procedure typically start responding after a fixed proportion of the interval has elapsed despite the absence of any external time cues. A widely-used discrete-trial variant of FI procedure is the peak-interval (PI) procedure. In the PI procedure, a stimulus such as a tone or light is turned on to signal the beginning of the to-be-timed interval and in a proportion of trials the subject's first response after the criterion time is reinforced. In the remainder of the trials, known as probe trials, no reinforcement is given and the stimulus remains on for about three times the criterion time. The mean response rate over a very large number of trials has a Gaussian shape whose peak measures the accuracy of criterion time estimation and the spread of the timing function measures its precision.
In addition of being accurate, interval timing is scale invariant: the time-estimation errors are proportional to the estimated duration. The origin and mechanisms of this fundamental property are unknown. We discuss the computational properties of a circuit consisting of a large number

of (input) neural oscillators projecting on a small number of (output) coincidence detector neurons, which allows time to be coded by the pattern of coincidental activation of its inputs. We showed analytically and checked numerically that time-scale invariance emerges from the neural noise. In particular, we found that errors or noise during storing or retrieving information regarding the memorized criterion time produce symmetric, Gaussian-like output whose width increases linearly with the criterion time. In contrast, frequency variability produces an asymmetric, long-tailed Gaussian-like output, that also obeys scale invariant property. In this architecture, time-scale invariance depends neither on the details of the input population, nor on the distribution probability of noise.

Disclosures: P.G. Lynn: None. **D. Novo:** None. **S. Oprisan:** None. **C. Buhusi:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.13/NNN52

Topic: G.06. Computation, Modeling, and Simulation

Support: BSI Director's Fund FY2009

Title: Estimating a network structure that underlies partially observed neuronal signals

Authors: *M. KOMATSU[1,3], J. NAMIKAWA[2], Z. C. CHAO[1], Y. NAGASAKA[1], N. FUJII[1], K. NAKAMURA[3], J. TANI[4];
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Abstract: Many existing studies have proposed various methods to quantify the interaction of neuronal signals. These methods were developed to evaluate the interaction among recorded signals in an isolated network. However, in order to obtain a more complete picture of information processing in the brain, we need to incorporate the influence from unobserved dynamics. In this study, therefore, we focused on revealing these unobserved dynamics, as well as causal interactions among observed neuronal signals. At the beginning, we proposed a variant of recurrent network model that consists of observable and unobservable units. The observable units represent recorded neuronal signals and the unobservable units are introduced to represent unobserved dynamics underlying the recorded signals. Connective weights, which indicate intensities of interaction between the units, could be estimated by an iterative learning with a maximum likelihood method. Then, we applied the model to 32- and 64- channels

electrocorticograms (ECoG) recorded from cortical surface of monkeys A and K, respectively. For each monkey, estimations of connective weights were conducted with four different data sets, and five initial learning states were tested on each data set. First, we explored connective weights between observable units representing ECoG by using a cluster analysis with Ward's method. In both monkeys, we obtained the consistent results: by introducing 5 unobservable units, observable units were divided into clusters, which were corresponded to anatomical cortical regions plausibly. Second, we investigated connective weights of unobservable units. We also found that majority of networks (12/20 of monkey A, and 18/20 of K) obtained from iterative learning were consistent. That means the unobservable units involved in the networks have similar influence on observable units. The results suggest that our presented network model is capable of revealing structure underlying multi-channel signals obtained from the brain.

Disclosures: M. Komatsu: None. **J. Namikawa:** None. **Z.C. Chao:** None. **Y. Nagasaka:** None. **N. Fujii:** None. **K. Nakamura:** None. **J. Tani:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.14/NNN53

Topic: G.06. Computation, Modeling, and Simulation

Title: An efficient finite difference approach to solving the time-fractional diffusion equation

Authors: *N. BHATTACHARYA, G. A. SILVA;
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Abstract: In recent decades, fractional differential equations (FDEs) have been used to model complex transport phenomena, including instances of non-classical diffusion, which experimental evidence and computer simulations suggest may occur in many biological contexts, such as white matter fiber tracts, dendrites, cell cytoplasm, and in the neuromuscular junction. Finding tractable analytical solutions to FDEs is difficult and in many cases not possible, and therefore the development of stable and accurate numerical methods is vital. In trying to apply existing computational methods for solving the time-fractional diffusion equation, we found that there were tradeoffs between accuracy, stability, and complexity. We discuss the development of an efficient, 2nd order accurate, broadly stable numerical method for solving the two-dimensional time-fractional diffusion equation. We accomplish this by using the implicit Crank-Nicholson finite difference scheme to increase the stability regime of the method. In addition, we use an operator-splitting method and application of adaptive memory to reduce the complexity

and computational time needed to solve the time-fractional diffusion equation. Development of this numerical method now enables us to more efficiently use fractional diffusion to accurately model complex cases of diffusion in biology.

Disclosures: N. Bhattacharya: None. **G.A. Silva:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.15/NNN54

Topic: G.06. Computation, Modeling, and Simulation

Title: The investigation of reduction processing in FSCV using PPV

Authors: *D. KIM[1], Y. OH[1], H. SHIN[1], I. KIM[1], C. KIMBLE[2], K. BENNET[3], K. LEE[4], D. JANG[1];
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Abstract: Background subtraction fast-scan cyclic voltammetry (FSCV) based on carbon fiber microelectrodes (CFM) has been recently utilized for the detection of neurotransmitter release in in-vivo brain due to sub-second temporal resolution. Compared to oxidation peak of dopamine (DA) in FSCV, reduction peak was few shad on the light in analytical studies although it has been used as one of representative feature of DA. The reduction peak of DA in FSCV always showed less than oxidation peak. There are several reasons for this phenomenon, but, as far as our knowledge, there are few systemic studies for the reduction processing in FSCV. Paired-Pulse Voltammetry (PPV) was suggested in order to minimize the confounding factors such as pH changes and transient effects at the CFM surface in our previous study. The waveforms are paired in doublets, two identical waveforms (“pulses”), separated in time by an arbitrary but short interval at the holding potential. When the voltammogram for one the pulses comprising a doublet is subtracted for the voltammogram for the other pulse, the effects of pH change, for example, can be eliminated. Although PPV was proposed for differentiating complex analytes, in our thought, it could be appropriately employed for the examination of reduction processing of DA in electrochemistry. In this study, the reduction processing of DA in FSCV was investigated in depth with PPV by changing gap time and with various experiment conditions such as pH changing, scan rate, and peak potential of waveform. The results of our study demonstrated that the reduction may occur between scans at a holding potential and the reduction processing may

be rate-limited by the concentration of H+ at the surface of CFM. In addition, using this reduction processing mechanism, we could improve PPV waveform and might allow for more precise determinations of kinetics of neurotransmitter in in-vivo conditions.

Disclosures: D. Kim: None. **Y. Oh:** None. **H. Shin:** None. **I. Kim:** None. **C. Kimble:** None. **K. Bennet:** None. **K. Lee:** None. **D. Jang:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.16/NNN55

Topic: G.06. Computation, Modeling, and Simulation

Support: This work was supported by the Research fund of Survivability Technology Defense Research Center of Agency for Defense Development of Korea (No. UD120019OD)

Title: Finite elements ear model for sound transmission simulation

Authors: *Y. PARK, D.-P. JANG, I. KIM;
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Abstract: Introduction : The sound is transmitted through the pinna, ear canal, eardrum, ossicles, and cochlea. The ear structures also transform the sound signal by acting as a filter. The power and phase of output sound signal, transmitted by the ear structures, is different with input sound signal. Because of this, the dynamic model that mimics the shape and physical characteristics of the ear is important for knowing the signal that is transmitted to auditory nerve. The purpose of this study is to make a finite element ear model and simulate the sound transmission from the ear canal to the stapes footplate.
Methods : The geometry of the ear model was based on the micro computed tomography images. The material properties of various parts of ear were obtained from the previous studies of middle ear model, and modified to calibrate the vibration modes of tympanic membrane and stapes footplate similarly with experimental data. The finite element ear model was developed using COMSOL multiphysics. We solved the acoustic-structure interaction problem using a frequency domain solver of COMSOL multiphysics.
Results : The vibration modes of tympanic membrane and stapes footplate are similar with experimental data. The displacements of tympanic membrane and stapes footplate maintain almost constant up to 1 kHz and have peak values at around 1 kHz. At the frequencies above 1

kHz, the displacement decreased with an increased in the frequency. Figure 1 shows the vibration modes of tympanic membrane and stapes footplate.

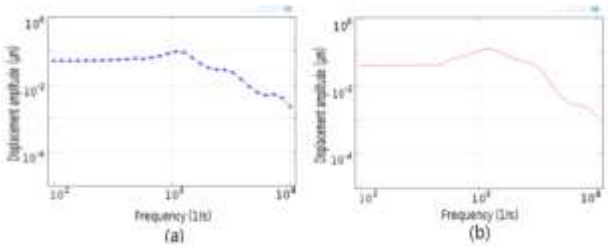

Figure 1 The vibration modes of tympanic membrane(a) and stapes footplate(b) at 80 dBSPL

Disclosures: Y. Park: None. **D. Jang:** None. **I. Kim:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.17/NNN56

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH R01 GM104987

Title: Optimal stimulus waveforms for controlling the behavior of a neuron: Gradient-based analysis reveals multiplicity of solutions

Authors: *J. CHANG[1], D. PAYDARFAR[1,2];
[1]Univ. of Massachusetts Med. Sch., Worcester, MA; [2]Wyss Inst. for Biologically Inspired Engin. at Harvard Univ., Boston, MA

Abstract: Many medical therapies revolve around the concept that an external stimulus (electrical, chemical, mechanical, etc) can bring a diseased biological system back into its normal state (e.g. cardioversion, defibrillation, deep brain stimulation). Unfortunately, many of these therapies have the potential to be just as detrimental as they are beneficial if too much of the stimulus is given. In this study, we seek to find the optimal electrical stimulation necessary to elicit a change in state within a neuronal model while minimizing the energy usage using a gradient-based algorithm.
In this paper, we show 1) the benefits of using a gradient-based algorithm to solve these optimization problems compared to more traditional techniques, 2) the phase-dependent nature of locally optimal waveforms, 3) the convergence of this algorithm in models with oscillatory states towards multiple locally optimal solutions and 4) the change in resonance properties of the

optimal waveform when presented with neurons of different latent excitability. This last finding has implications on what models are chosen when finding optimality in different neuronal systems. While an integrate-and-fire model may be suitable for finding optimality in a low-excitability neuron (e.g. motor neurons), they fail to showcase the resonance that is present in high-excitability neurons (e.g. epileptic neurons). Thus, in choosing an integrate-an-fire model, there is potential for missing out on finding more optimal resonating solutions.
We also demonstrate that with the gradient-based algorithm, one can find optimal solutions given only partially defined terminal conditions. Thus instead of specifying complete terminal conditions, we can define only the terminal conditions that are experimentally relevant, removing artificial constraints from the optimization algorithm.

Disclosures: J. Chang: A. Employment/Salary (full or part-time):; University of Massachusetts Medical School, Wyss Institute for Biologically Inspired Engineering at Harvard University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH. **D. Paydarfar:** A. Employment/Salary (full or part-time):; University of Massachusetts Medical School, Wyss Institute of Biologically Inspired Engineering at Harvard University.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.18/NNN57

Topic: G.06. Computation, Modeling, and Simulation

Support: Taiwan I-RiCE Program NSC-100-2911-I-009-101

Title: Detecting different states of migraineurs based on resting eeg and ssvep habituation

Authors: *L.-W. KO[1], K.-L. LAI[2], S.-B. HUANG[1], M.-H. YANG[1], C.-T. LIN[1], S.-J. WANG[2]; [1]Brain Res. Ctr., Natl. Chiao Tung Univ., Hsinchu, Taiwan; [2]Neurolog. Inst., Taipei, Taiwan

Abstract: Migraine is a neurological disorder in the world. Approximately one billion people (15% of the total population) are suffering from this disorder. Migraine characterized by episodes of severe headache, which is often pulsatile in nature and unilateral in location. Attacks are usually associated with nausea, vomiting, and sensitivity to light, sound, or movement. From clinical point of view, an altered state of brain excitability may be the fundamental pathomechanism, which render *migraine* patients more susceptible to external stimuli. The posterior mean dominant frequency was significant reduced during the attacks, with a significant

increase of interhemispheric asymmetry. These studies evidence the migraine as a neural system dysfunction. Other electrophysiological studies have addressed the issue on the brain excitability in patients with migraine, including somatosensory, visual and motor cortices by somatosensory evoked potential (SEP) and visual evoked potential (VEP). These studies show that two general phenomena exist in migraine patients: first one is central hyper excitability in SEP studies and the other is habituation defect (dis-habituation) in VEP studies, which make them more prone to headache development. This study is related to VEP and resting state of patients. The main goal of this study is to observe the habituation and resting power changes of the migraine patients and aim to find an indicator to classify the patients into three different states of migraine (i.e. inter-ictal, pre-ictal, and ictal).One hundred and ten patients in different migraine states and 9 control subjects performed the steady-state visual evoked potential experiment with EEG recordings including a series of visual stimulation at various flashing frequencies, in which 13 Hz flashing was repeated twice. The states of migraine were determined according to headache diaries. Patients who did not have migraine attack within a period of 36 hours before and after the EEG recording were classified as inter-ictal state. Those with migraine attack within 36 hours before and after the recording were classified as post-ictal and pre-ictal states. The ictal state denoted those who had migraine attack upon examination.
In this study, we applied closed-eye resting EEG and fluctuant habituation pattern to classify different migraine states. The results indicated that the resting EEG and the change of habituation can be used a reliable neurophysiological hallmark of migraine. It provides the clinicians the EEG predictors of migraine attack period. Then, this can assisted physicians to select appropriate medicine to reduce the suffering of patients.

Disclosures: **L. Ko:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NSC-101-2911-I-009-101, Taiwan National Science Council I-RiCE Program. **K. Lai:** None. **S. Huang:** None. **M. Yang:** None. **C. Lin:** None. **S. Wang:** None.

Poster

588. **Computation, Modeling, and Simulation VIII**

Location: Halls B-H

Time: Tuesday, November 12, 2013, 8:00 AM - 12:00 PM

Program#/Poster#: 588.19/NNN58

Topic: G.06. Computation, Modeling, and Simulation

Support: Sandia LDRD 151345

Title: A computable database for neural model generation

Authors: *F. ROTHGANGER, D. TRUMBO, C. WARRENDER, B. AIMONE;
Sandia Natl. Labs., Albuquerque, NM

Abstract: We are developing a software package called "Neurons to Algorithms" (N2A). The scientific purpose of N2A is to facilitate biologically realistic network model development by 1) compiling (and referencing) neural data from many users and sources, 2) representing this data in a computable format, and 3) automating the process of simulation and analysis on large-scale computers.

Many have endeavored to create a definitive central repository of neural and/or biological information. All of these collections contain descriptive data, and in theory any of them could be amenable to structural analysis. However, it is rare to find a collection that can translate directly to a simulation. That is the guiding principle of this tool. It represents both structural relationships and the explicit quantitative behavior of each part. All math is in the form of simple declarative equations that describe the system dynamics.

For a given simulation, the user selects a subset of parts from the database, and specifies the quantity and spatial arrangement of each one. N2A generates the full network and sends it to a simulator such as Xyce or Neuron. It then monitors the progress of the simulation and collects the results.

The tool has been designed to keep both private models and community-shared models, and to easily move between the two. Our vision is that this community database will enable the generation and sharing of high fidelity model parts across laboratories. Where possible, N2A will interface directly with other types of repositories to avoid duplication of effort.

We will demonstrate the N2A platform using a model of the hippocampus (Aimone et al, 2009).

Disclosures: F. Rothganger: None. **D. Trumbo:** None. **C. Warrender:** None. **B. Aimone:** None.